

Can, or should, the swing be stopped?

A MONTH ago we looked at the way that student preferences were changing in Cambridge. Now the Universities Central Council on Admissions has released a comprehensive statistical analysis of students' applications and admissions for all universities of the United Kingdom (Statistical Supplement to the Eleventh Report, £1.25). The figures apply to the year of admission 1973—it is some disappointment that it takes almost a year to produce a document of such importance—but the sections of most interest deal with trends since 1969.

Many of the figures are rock-stable from year to year. In 1969 11.6% of applicants came from the North West, in 1973 11.3%; 33% from the South East in 1969, 33.7% in 1973; and so on. The percentage accepted is uniformly 51% both in space and time. The only deviation seems to be Scotland which apparently can only get 40% of its applicant sons and daughters into universities. But then your instinct says that surely the Scots do not seem any more stupid than the rest, further hunting reveals that 'three Scottish universities are partly outwith the UCCA scheme' and your dictionary tells you what that word means and that it is of Scottish origin.

The middle classes, less than 30% of the population, provide almost three quarters of the candidates—and working class applicants have declined from 29% to 26% in five years, although they do now fare marginally better in getting accepted.

Whilst all these trends are slow or negligible, one trend has been dramatic: the swing away from science. In the figure we show the way in which fashions have been moving in seven subjects which have large enrolments at university. For instance, a value of +10% for mathematics for 1971 means that there were 10% more applicants for entry in 1971 than in 1970 who gave mathematics as their preferred subject. Some other subjects not shown here have comparable trends; physics and mathematics follow similar patterns, so do mechanical and electrical engineering. Geography, English and History are relatively static. Social, administrative and business studies follow strange ups and downs but have shown no long term trend.

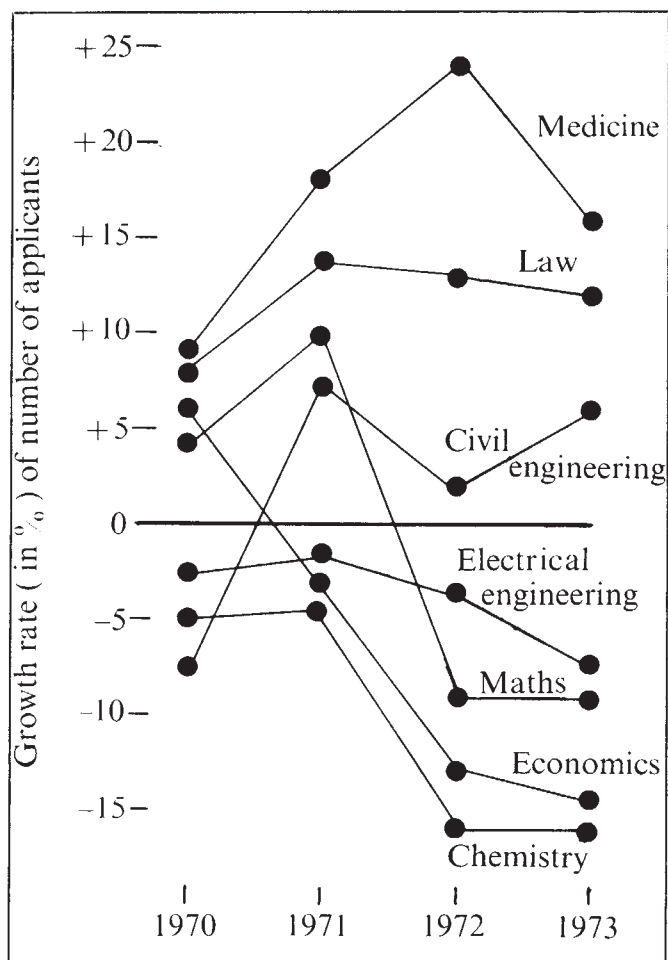
In terms of absolute numbers, there were 7,000 applicants for medicine in 1969, almost 13,000 in 1973; 5,000 for law in 1969, almost 8,000 by 1973. Chemistry on the other hand had slumped from 3,700 to 2,400. Less than one in four of those applying in 1973 to do medicine were admitted, whilst two thirds of those applying for physics or chemistry were accepted. What is more a substantial fraction were admitted to physics or chemistry who had not even listed it as first choice.

It used to be said disparagingly of medical students that they were only in medicine because they found physics

and chemistry too difficult, but figures in the UCCA compilation show that the increased selectivity now possible has made the entering medical undergraduate the best academically qualified of any.

Everyone will have his own explanations of these trends, but two things need careful underlining. The dramatic transition from the 1971 to the 1972 entry was no flash in the pan since the 1973 growth rates are closely similar to those of 1972. If science applications were depressed in 1972 by gloomy job prospects (up to six or seven years in the future!) and disenchantment with technology's environmental record, then to keep the growth rate so negative for yet another year would have required even more gloom and disenchantment.

Second, it is insufficient simply to acknowledge the falling off in science applicants, say yes it was bound to happen some time, hold a meeting or two and hope that the problem resolves itself somehow. The diversity of opinions expressed at the British Association last week (page 91)—good prospects in engineering, bad in biology cannot but confuse the school-leaver. The message from school science teachers is that there is no turnaround in recruitment to science in the immediate future. Can the country, can science afford to lose out in this way? The answer is not automatically that it can't, and it would be foolish blindly to assert that the number of scientists must always grow. Nevertheless at present the size of the annual intake is controlled by forces which are so remote from job prospects four to seven years hence that a market place type of situation could hardly be said to exist. Eventually the community must come to grips with the question of how it wants its future size determined.





Ominous news for marine science

The Law of the Sea Conference is over and the way in which marine scientific research is to be organised and controlled is not yet agreed.

CARACAS—the site of the Third United Nations Conference on the Law of the Sea—a booming, noisy city, set amidst the green hills of the national park area. Ten weeks of slow and protracted discussions in three main committees, each open to delegates from all the 137 countries attending, plus numerous intergovernmental and nongovernmental bodies of all sizes, composition and persuasion. Superb arrangements laid on by the Venezuelan Organising Committee, together with the United Nations Secretariat (at considerable cost) though a number of nations (particularly in Africa and Asia) expressed concern at the communication difficulties they have encountered and expressed their need to keep in close touch with their governments, particularly during the later negotiating stages of the conference. Chiefly for this reason it was decided to hold the next session in Europe.

Almost unanimous agreement that further sessions should not exceed six or seven weeks in length as humans get tired and stale if they attempt to confer for a longer period and little is achieved in the extra weeks. Winter troglodytes from northern latitudes trying to avoid another summer session—we have not had an opportunity to see our families in the sun for six years; smaller and less well off countries trying to reduce the length of future sessions, owing to the high cost of keeping large delegations abroad—countries have now had the opportunity to define their positions, let us get on with the negotiating and this will only happen during the final session; other countries talking about two sessions in 1975 and at least one in 1976.

Countries with differing views on the importance of the whole exercise—some suggesting that this was the most important conference that has ever taken place on the earth, and that we were trying to save civilization; others wondering whether the conference had the slightest relevance to the future of the human race; and still others believing sincerely that it was doing positive harm to the aspirations of the less developed countries.

Sharp disagreements within delega-

tions, usually ending up with victory for military and economic interests over those of the marine scientists and environmentalists. Different types of disagreement between marine scientists—one faction claiming that freedom of scientific research in the oceans is essential for the future of scientific research as a whole, and that the restrictions proposed would either kill marine science dead or make it an undercover activity which no individual state or international authority could hope to prevent; or the opposing view—that developed countries have got to realise that the days of 'Challenger' type expeditions at the expense of the natives are over, and it is now the turn of the developing countries.

These may be negotiating positions, of course, and doubtless if the declaration made by the President and endorsed by the conference on June 27, is to have any effect, the extreme views will disappear in the interests of gaining concessions elsewhere. This declaration reads:

"Bearing in mind that the problems of ocean space are closely interrelated and need to be considered as a whole, and the desirability of adopting a Convention on the Law of the Sea which will secure the widest possible acceptance, the conference should make every effort to reach agreement on substantive matters by way of consensus and there should be no voting on such matters until all efforts at consensus have been exhausted."

This consideration is in fact vital when it is remembered that the first UN Conference on the Law of the Sea, Geneva, 1958, which produced four international conventions, has received relatively poor support from states. These were the Convention on the Territorial Sea and Contiguous Zone (46 states parties, in force since 1964); the Convention on the High Seas (54 states parties, in force since 1962); the Convention on Fishing and Conservation of the Living Resources of the High Seas (35 states parties, in force since 1966); the Convention on the Continental Shelf (53 states parties, in force since 1962). Furthermore, the second UN Conference on the Law of the Sea, Geneva, 1960, failed in its attempt to resolve disagreements over the breadth of the territorial sea and fishery limits.

The three main committees inherited the work of the UN Committee on the Peaceful Uses of the Sea-Bed and the Ocean Floor beyond the Limits of National Jurisdiction and it is on the lengthy deliberations of that committee

over a period of five years that the present discussions are based and the texts of the convention articles will be developed.

It is becoming clear now that the final agreement will most likely include a territorial sea twelve miles in width, an economic zone beyond the territorial sea (referred to by the Latin Americans as the 'patrimonial sea') and an international zone covering the deep sea-bed, the water column being high seas. The general trend embodying several proposals is that countries where the width of the shelf is less than 200 miles, will have an economic zone of 200 miles covering both the shelf and the water column; others, where the shelf extends to over 200 miles, will have an economic zone of 200 miles and in addition sovereignty over the sea-bed out to the edge of the continental rise (this additional sovereignty will not include the water column). The fact that this limit is unidentifiable in most parts of the world because of the lack of data or lack of rise, has so far been conveniently ignored.

Among the more controversial matters on which as yet no agreement is in sight, are the extent of coastal state jurisdiction in the economic zone with regard to such matters as pollution control, scientific research, exploitation of living resources; and the degree of control to be exercised by the international authority over the international sea-bed area—this varies from the "none" of the states most physically capable of working in this area to the vesting of exclusive and direct control of all exploration and exploitation in the International Authority. A serious draft article "Right to undertake Marine Scientific Research", at present on the record, states "Marine scientific research in the international area shall be conducted directly by the international authority and, if appropriate, by persons, juridical or physical, through service contracts or associations or through any other such means as the International Authority may determine, which shall ensure its direct and effective control at all times over such research".

The fear of scientific research shown by many of the developing countries is based on two factors, one real and one controversial. The former is the military bogey, the possibility that a ship operating off the coast of another state may be gathering data inimical to the coastal state, in addition to its declared programme (and not necessarily oceanographic data; for example the

Pueblo episode has been invoked on more than one occasion). In fact this is a case of the past behaviour of certain countries with regard to the release of oceanographic data coming home to roost. The theory behind the present World Data Centre system is that each country by injecting its data into the system qualifies to extract all data sent in by other countries. In fact a number of countries are notorious in that they participate in the system but, caught by military classification of data, they submit a minimum of their own, at the same time however calling loudly for complete submission of all data by others. This is particularly true of water temperature and to a lesser extent salinity data, of use to the military for acoustic detection of submarines. Marine pollution data, being of little military value, escapes the military clampdown.

The counter arguments deployed are that no convention or international regime is going to stop this type of behaviour. The navies of the world will continue with this policy until submarines or sonar become obsolete and it is the marine scientists wishing to work on other pressing problems who suffer.

The second fear is economic and is based on the premise that knowledge is power—in some unexplained way knowledge of the sea or sea-bed gained by a country carrying out scientific research, can be used to its economic advantage at the expense of the coastal state. In fact the coastal state desperately needs such knowledge if it is to benefit from any worthwhile exploitation of the area over which it has rights, and illegal exploitation which is a much slower process, will be far easier to prevent, particularly if the International Authority were developed to this end and the world's navies, which after all are the only possible forces that can police these areas, are mobilised in support.

At the present, the International Authority is seen by many countries as a panacea for all ills. There has been complete lack of consideration given to the way in which the authority will carry out its terms of reference and the structure it will need for this purpose, that is, how it will assess research projects submitted to it, whom it will recruit to carry out this task, what they will be looking for anyway. In theory, the authority will have to study each project submitted to it and to decide that the declared objective or purpose of the research is as stated, that it is not to the detriment of any coastal state (if in waters over which it has right) and that other criteria are laid down are being adhered to.

To undertake a task of this nature, a very large staff of highly qualified

marine scientists of many disciplines would be needed and it is quite clear that inherent delays in obtaining consent would be enormous. At the same time that the developing countries are asking for the international authority to take on this vast and sophisticated task, they are clamouring for a transfer of technology, by which is meant an expanded training and education programme to build up their national marine science infrastructures. This latter exercise is fully justified but could well be killed by the former. But in any event very little detailed or serious consideration has been given to this subject and the conference has before it only one very general proposal dealing with the matter.

The basic world shortage of marine scientists in all but a few countries has

“It is a sad reflection on our times that . . . most delegations present consisted of lawyers and politicians, with little or no scientific support”

led to a considerable reluctance by the United Nations and its specialised agencies to recruit top people in this field from the developing countries, as this usually results in an immediate weakening of the marine science infrastructures in the countries concerned; almost certainly these have been painstakingly built up over a number of years.

The staff, for a viable international authority capable of carrying out the assessment tasks proposed for it, would have to be obtained either from the very developed countries whose activities the authority has been set up to restrain or if, as is more likely, the United Nations staff quota system is used, largely from the developing countries; the latter arrangement would spell disaster to the marine aspirations of many of those countries.

There is also a real fear that the international authority will become yet another United Nations agency, to be paid for by the developed countries, that is, those countries whose activities will be most heavily constrained, with a complete lack of internal policy except to increase its empire and to become operational at the expense of other bodies which have been working in the same field, often for many years. Existing United Nations bodies already have a good if inadequate record of activity in this field; the Intergovernmental Oceanographic Commission of Unesco, for instance, already offers its member states an independent assessment facility for any project in

which there is cause for dispute between a coastal state and a state wishing to carry out scientific research in waters over which the coastal state claims jurisdiction.

A fruitless exercise at present being carried out by certain of the developed countries, is an attempt to legislate separately for pure and applied research, as if these are rigidly definable activities. Certainly there is a white area of pure marine scientific research such as investigation of the history of the earth and the theory of plate tectonics; certainly there is a black area of applied research such as development of seismic survey methods to detect salt domes capping oil reservoirs, thus providing an indication to the oil companies of the most likely positions in which to drill to obtain a strike; but in between, there is a great grey area having both pure and applied connotations inextricably mixed, and anyway there is very little pure research that does not have an applied fallout of some kind. Besides this, such legislation would do considerable harm to applied research and survey work, needed by both developed and developing countries alike if the ocean resources are to be exploited economically for the benefit of mankind.

An unfortunate trend that has evolved during the Sea-bed Committee and conference sessions, has been the apparently successful attempt to lump all sorts of diverse data collecting activities under the heading of scientific research, which of course they are not and never have been; these include meteorological, oceanographic and bathymetric data gathering for such purposes as weather forecasting, compilation of navigational charts and so on. For instance, if a country wishes to place automated transmitting buoy systems (known as ocean data acquisition systems, aids and devices, or ‘ODAS’) off the coast to report the approach of hurricane force winds, this is now known as scientific research.

It is essential that such activities should be seen in their true perspective as ocean services—activities for the compilation of products for the benefit of developed and developing countries alike. Restrictions on data collection and exchange can help no one and do harm to many.

It is a sad reflection on our times that although, as pointed out by the Secretary of the Intergovernmental Oceanographic Commission to Committee III, a basic knowledge of the oceans—what they are, how they behave, what they consist of, and so on—is essential to the success of the conference and the subsequent convention, yet most delegations present consisted of lawyers and politicians, with little or no scientific support. □

international news



THIS year's summer meeting of the British Association (BA) in Stirling, four days of lectures devoted to science ranging in purity from the geology of Scotland to witchcraft, was deemed a great success by its retiring President, Sir John Kendrew. More than 2,000 members attended this unique forum, the best attendance for seven years.

Dr Magnus Pyke, Secretary of the Association and Chairman of Council, (pictured above) sees the BA as a "peripatetic university; it obtains its strength from movement". Certainly, to judge from those in attendance, it seems to recruit many of its officers and much of its following from towns which have been host to the association in the recent past. The ambience does seem to be very important and was a major factor in the success of this year's venue, as a result of "the intellectual richness of Scotland".

There was no feeling of nostalgia for the BA meetings of the past, at which many new results were communicated. Sir John made it clear that though original work would be very welcome, "the process of communicating science has changed completely since the early days of the BA". Dr Pyke feels that the function of the BA is to communicate a policy of social responsibility. Dear to Dr Pyke's heart are the working parties and their reports. But (as the reports reflect) Dr Pyke is adamant that the association should not adopt a radical role. "We don't want to tear down ICI" he told *Nature* vehemently. "We are here to make the facts clear;

The British Association at Stirling

period." He stressed that it is the aim of the BA to educate members of the public so that they will be placed in a position from which they can exercise their rights as informed voters.

But it is interesting to consider whether those present really constituted a random section of the non-scientific public that Dr Pyke is trying to reach. The meeting, and the publication of the report on Biomedical Advances that had preceded it, received a fair amount of press coverage and Sir John felt that there had been an encouraging amount of responsible reporting. Without doubt the BA has been actively promoting itself in the recent past and at last seems to have realised that it is the only way that it can reach the wide public audience with whom it claims to communicate.

There was certainly an age gap in those attending the meeting. Most were either under 25 or over 50 and the small middle-aged group of active scientists seemed to be there to organise or to speak. Schoolchildren seemed much in evidence, both organised and disorganised, and some of the more fortunate undergraduates and schoolchildren had managed to get grants from their local

education authorities. In addition of course there were teachers, government and industrial scientists (with a sprinkling of academics) and the mild elderly ladies who go each year simply for enjoyment.

In fact, schoolchildren seem to be the part of the population which the BA reaches most effectively. Young members are joining local BAYS groups at a rate which is beginning to cause administrative headaches, and Dr Pyke wryly admitted that the situation was becoming embarrassing. Many members of Council, notably Sir Lincoln Ralphs, are enthusiastic about the interest shown by the young, but Dr Pyke himself has been criticised by both the youngsters themselves and by many BA officials, for his lack of interest in the juvenile membership. There was certainly little provision for them at this year's conference.

Whilst it must be admitted that Dr Pyke, in his role as Chairman of Council, has provided an immensely important element of continuity in the organisation of the BA, it is sad that his enthusiasm does not seem to extend beyond his individual view of the current welfare of the association. There will come a time when, like it or not, Dr Pyke and his generation will have to leave and it would be as well to prepare for that contingency. It would be a pity if, after the successes of the current resurgence, the BA has to spend yet another period in the doldrums for lack of encouraging their future membership now.

If you want to be a research biologist, a degree can be a hindrance, according to Professor Kenneth Mellanby, former Director of Monks Wood Experimental Station. Addressing the British Association, he said that promising school leavers now stand a better chance than the average graduate of a career in biological research.

The career structure of research institutes, especially those financed by government, favoured two types of scientific worker. There would always be room for the first-class graduate with a higher degree, although the number of such vacancies, already small, may decline even further as the Fulton proposals improving promotion prospects for junior staff come into force. At the other end of the scale, said Professor Mellanby, most Directors prefer bright school-leavers for junior research and technical posts, and there is no lack of these wishing to take up a biological career.

Although Professor Mellanby acknowledges the situation he is far from happy about it. If good graduates and postgraduates cannot get jobs in research the quality of research will inevitably suffer, he said. In his view, the scientist who had risen through the ranks, admirable though he or she might be at the job, would not have a similar capability for research.

Professor Mellanby's impression was of a "great over-production of graduates and doctors and high unemployment". But, in fact, after an initial disappointment, the biology graduate usually found a congenial niche in a variety of other occupations.

But industries requiring graduates with a specialised applied training such as chemical engineering or metallurgy are experiencing a shortage of trained graduates to fill an ever increasing number of jobs. In 1974 the number of

applications for degree places in chemical engineering dropped dramatically at a time when the demand for chemical engineers had suddenly taken a rapid upwards turn. The number of jobs in chemical engineering advertised in the Institution of Chemical Engineering's 'Diary' had dropped from 742 in 1969 to 178 in 1972 but had risen to 405 in 1973 and in 1974 was

Job crisis or manpower crisis?

by Eleanor Lawrence

expected to top the 800 mark, said Dr David Sharp of the Institution.

Was this decline in graduate applications simply a result of straightened job prospects during the early 1970s? Dr Sharp is convinced that the cause lies much deeper; physical science teaching at secondary school level is neither inspiring enough nor of sufficiently high quality to encourage the school child in this sort of career he said. No graduates with engineering backgrounds went back into teaching at secondary school level, so that schools careers officers were completely ignorant of courses and career prospects. The tiny proportion of physics graduates entering teaching also worried Dr L. Cohen of the Institute of Physics. Chemistry may fare better, as in 1973 about 15% of first degree chemistry graduates entered teacher training.

Industry would no doubt find ways of getting round the specific manpower shortage, said Dr Sharp. The slack would probably be taken up by people trained in related disciplines such as chemistry. But a shortage of metallurgists could hold up programmes of

metal reclamation and recycling said Dr D. C. Moore, President of the Institute of Metallurgists, as university departments of metallurgy declined and in some cases disappeared.

So the "job crisis" tends to look rather different depending on where one is standing. A picture emerges, not so much of a crisis, but of increasing diversification amongst science graduates. Everyone was agreed that, except in the special cases of the applied fields, there certainly has been a drop in jobs which use scientific training directly. Some parts of industry had increased their recruiting this year, but in the present financial situation, the universities and the scientific civil service would take fewer and fewer people.

But the patterns of first year employment seem to be evolving to meet the changes quite successfully, though how much individual disappointment is hidden behind the statistics is hard to tell.

Diversification is not necessarily a forced choice. Salaries of professional scientists compare very unfavourably with their contemporaries in management or administration in both industry and the civil service. Dr R. E. Parker of the Royal Institute of Chemistry produced comparisons of salaries between research, technical and management grades in the chemical industry which clearly direct the financially ambitious away from pure science.

Employment or lack of it amongst science graduates must be set in the wider economic context. Dr Cohen emphasised that absolute unemployment amongst science graduates was still lower than amongst graduates as a class. And it was pointed out more than once that other graduates had always been in the position of having to find work unrelated to their specific training.

Only connect

by Gillian Boucher

SCIENCE undergraduates lack the critical training needed to see their subject in the broader context of society or even of science as a whole. This is the thread running through the many experimental curricula and proposals for change described during a week-long session at the BA. Motives and methods varied widely but the courses described fitted into two broad categories: traditional science taught alongside 'science studies' (the salad principle); and the interdisciplinary science course (the porridge or porridge principle).

Dr David Edge, Director of the Science Studies Unit in the University of Edinburgh, described the alternatives to the traditional British subject-

oriented course. First, a course aiming to fit the student for a career. This might entail teaching an additional subject like economics, or a skill like decision making. Second, the course could be more geared to the personal needs of the student who may feel that his development requires more than a single science taught in a particular way. Third, courses could aim to make students of more immediate use to society as a whole.

There was complete agreement that the traditional science courses and subjects should remain on the curriculum. But there was strong support for the provision of alternative courses. The commonest reasons given were the interests of the student's future career and of society, the two often being treated as identical. In the eyes of Pro-

fessor F. R. Bradbury of the University of Stirling the 'Advancement of Science' was no longer so much in the public interest as it had been when the British Association was founded: instead, students should learn to manipulate the scientific knowledge already attained and apply it to the messy problems of real life.

Those who preach interdisciplinarity are adamant that the porridge should be smooth. It is not enough to teach several subjects side by side as in a joint honours degree or the old general degrees. It is not even enough to allow the student a great deal of choice between course units. Integration requires a totally new approach—an emphasis on the connections between subjects, which will lead to the subjects themselves being taught differently. To

THE BRITISH ASSOCIATION AT STIRLING

create such a course is very difficult—Dr Edge stressed the danger of a superficial juxtaposition of subjects. This can only frustrate the thoughtful student who wants to read further and discovers no core of knowledge and research below the superficial link.

Most of the interdisciplinary courses in existence in the United Kingdom—for example those at Aston and Stirling Universities and the Middlesex Polytechnic—teach two or more traditional subjects and attempt to link them by means of problem-solving courses. In these students are presented with a real life situation and asked to consider all the relevant knowledge from the various disciplines in coming to conclusions. Thus the student develops a skill which is not demanded by a traditional course, and is presumably more personally fulfilled and more ready to pursue a career and be of use to society. Sometimes even more is asked of these subject-spanning courses—they are

supposed to provide the basic framework for the study of the traditional sciences. Thus chemistry and cell biology are taught in the context of a marine pollution course at Stirling. Clearly the links in many cases are fragile but perhaps for some students the applicability to social problems is enough.

There are other dangers of which, says Dr Edge, the proponents of the new courses may not be fully aware. In a subject-oriented course with rigid boundaries between subjects, the teacher is in firm control of what the student learns and can withhold from him the realisation of the gaps and ambiguities in knowledge until near the end of the course. All this makes for a very clear relationship between teacher and taught, with the teacher in charge. Changing to an interdisciplinary course will remove subject loyalties and may disorient both teacher and student. The teaching of a subject

as part of an interdisciplinary course will entail teaching its fundamental concepts at a much earlier stage, and omitting much of the detailed knowledge of the subject. This will tend to diminish the traditional status of the teacher which again will be disorienting to all concerned and will be fiercely opposed.

Some of these problems have already been encountered by those teaching the new courses. Dr Edge's views on the relative power of student and teacher would not be accepted by all—seemingly Professor Jevons of the Department of Liberal Studies in Manchester feels that the student doing a course on marine pollution was in fact far more at the mercy of his chemistry teacher than a student doing a traditional chemistry course. The problem of subject loyalties may be solved by providing teachers and students of interdisciplinary courses with an institution which has some of the trappings of

SCOTLAND'S economic salvation need not be entirely dependent on oil.

The quarrying industry in Scotland could soon be in a position to gain a substantial share in a market worth £2,000 million a year, which could have a considerable impact on the British economy, it was claimed in a lecture to the British Association. "It is fashionable to look on oil as the saviour of our economy", said Dr Colin Gribble, a geologist from Glasgow University and Mr Ian Wilson, an executive with Amalgamated Quarries (Scotland) Limited; but they claimed that, with the adoption of a scheme entailing the low-cost transportation of bulk material, high quality granitic material could become as important as oil in the longer term, both on the home and export markets.

Quoting government statistics, they said that by the end of this decade the annual demand for roadstone and construction material in Great Britain will have risen by 50%, so that an extra 100 million tonnes of aggregate will be required each year. The increased demand is likely to be particularly high in south-eastern England, a densely populated area already using a high proportion of the British annual production. Materials there are either very scarce or completely exhausted, and most aggregate is supplied from Cornwall, Leicestershire, and Wales. But the largest of these quarries, said Dr Gribble, can only produce about 2 million tonnes a year, and they do not have the reserves to allow for the increased output which will be necessary to meet

the predicted growth in demand.

"We believe that Scottish quarries are in a position to supply a significant share of this demand. We also believe that we have the technology to supply that demand at competitive prices",

Scotland's future in the rocks

by Allan Piper

said Dr Gribble and Mr Wilson. Their calculations depend upon the assumption, reasonably based on an extrapolation of present trends, that the cost of aggregate in 1980 will be about £10 for a delivered tonne at today's prices.

All that is required, they argue, is a novel modification of the present system of quarrying. They suggest that massive quarries, with reserves of over 100 million tonnes, should be developed in remote, granitic areas close to the coast. Each quarry would be worked to produce about 10 million tonnes a year. Such massive reserves, coupled with the intensive output, would reduce the cost of land fees, manpower, and plant renewal, and the consequent reduction of necessary capital investment would render the industry extremely cost-effective, it was claimed. And the location of the quarries near to the sea, preferably close by deep-water harbours, would provide easy access to a convenient and relatively economical means of bulk transportation. Labour

requirements should provide little problem, according to Dr Gribble, as only about 100 men would be required to work a quarry of the envisaged size.

"Sites for these very large remote quarries would be carefully chosen and landscaped, screened from view, and so on," said Dr Gribble. He suggested that the development of 10 to 20 such quarries, located almost entirely on poor agricultural land, might be more desirable environmentally than the continual opening of smaller quarries in populated areas as the demand arises.

Mr Wilson's company already has two quarries on the east coast, at Peterhead and Inverkiething, which, it was claimed, could easily be developed on the proposed scale once the market became ready. And Mr Wilson knows of at least two other quarries in Scotland, which are in a similarly advantageous position.

The quarries at Peterhead and Inverkiething are, according to Mr Wilson, already capable of supplying material by sea not only to south-eastern England, but also to northern Europe where there is a similarly increasing shortage. He is quite confident that, if the suggested ideas are implemented, Scottish quarries could eventually provide as much material for export as for the home market; "we already have customers in Germany and France anxious to buy this material from us", he said. Both he and Dr Gribble are optimistic about the future of the Scottish quarrying industry which they believe could considerably bolster the future economy of Britain.

a department. A further problem is a shortage of reading matter. SISCON—the one-year-old Science in a Social Context project—is trying to fill this gap. A group of eight universities and one polytechnic running science studies or interdisciplinary courses have been given £50,000 by the Nuffield Foundation to produce course books which are flexible enough for a wide variety of courses. Dr W. F. Williams, the SISCON project coordinator, sees the job as essentially short term. The aim is to produce as wide a range of teaching material as possible and if within ten years this is not being supplemented and replaced by books written outside SISCON the whole idea will probably have been a failure. Dr Williams is also toying with the possibility of sending a researcher to Nigeria to gauge the need for similar publications in Africa.

And what of the courses that do not attempt to break down the traditional boundaries? Dr Edge's institute in Edinburgh provides only one quarter to one half of a student's work for one year. Dr Edge aims to give students an awareness of science—its internal behaviour and its relations with the world—without taking up so much of his students' time to prevent them from progressing to scientific research if they wish.

Professor Jevons's department in Manchester similarly offers a single unadulterated science alongside science studies. But the entire course—science and science studies—is within a single department and Professor Jevons does not aim to produce scientists ready to do research. The students only spend half their time doing science and the other half looking at science in its social, historical and economic context.

Undergraduates prefer to be taught distinct subjects rather than a homogenised baby food, said Professor Jevons. It would be a pity to break down the real outlines of the traditional subjects in order to erect the flimsy bridges between the interdisciplinary courses. Yet students want to participate and debate and learn to take decisions. Since the well-worn precepts of the physical sciences are patently unsuitable material for this style of teaching, the best solution is to teach students something else besides their science. In Professor Jevons's department this something else is science studies, which can be taught by science teachers. This part of the curriculum develops the student's critical faculties which are by necessity blunted in the acceptance of scientific truths. The student becomes more aware of the role of science in the world and perhaps even a better scientist.

A final word of warning against the excesses of interdisciplinarity. Professor Kendrew reminded the meeting

As a rest from the round of lectures and seminars, British Association members had the opportunity to look around Redpath Dorman Long (North Sea) on the Fife coast. The little village of Methil on the coal coast of Fife once boasted a thriving colliery but since the 1960s unemployment had increased and the area had begun to show the inevitable signs of industrial dereliction.

But in 1972, RDL (North Sea) set up at Methil, on the disused 136-acre colliery site, when the company was formed from a consortium of British and Italian interests. RDL, a subsidiary of British Steel, hold a 55% increase and the remaining 45% is held by three Italian companies with long experience in the design and construction of oil production platforms in the Mediterranean and the Middle East.

RDL's first steel jacket, for the Auk field, was successfully placed in position on its 280 feet deep water site this summer, and RDL are now building an even bigger structure for the 480-foot deep Brent field. As the weight of the structure varies with the square of the depth of the water, this platform is twice as high and four times as heavy as the 4,000 ton Auk platform. RDL have also started work on a comparatively tiny gas production platform for the Danish Dan field.

The site at Methil could certainly cope with at least one medium and one large-sized jacket at the same time, said a company spokesman. In this RDL think they have an advantage over some of the other steel platform con-



Work returns to Methil

structors, who use the flotation tank method for building and floating out platforms and thus can build only one at a time. RDL build their platforms on dry land and then manoeuvre them onto submersible barges which carry them out to the site.

The Methil site has only 30 feet of water for floating out but this is perfectly adequate for the steel jackets which are towed out on their sides. RDL also have on offer a steel/concrete hybrid gravity platform but as yet have no takers. This design can also be built at the Methil yard and is suitable for water depths up to 600 feet, rivalling the concrete platforms.

that certain "hard" subjects such as mathematics had to be learnt young or not at all. The early molecular biologists showed that it was a positive advantage to enter a field of which you had no previous knowledge, as long as the basic mathematical ability was there. And certain subjects could very properly be picked up by the student or graduate when he felt a need for them—witness Professor Kendrew's success with crystallography. □

Social concern

MRS Shirley Williams, Secretary of State for Consumer Affairs, drew the attention of a British Association symposium to the difficulties that politicians find in keeping in touch with science. "When I was a minister with responsibility for science", she said, "I found that ministers with such responsibilities are not in touch with scientists". Speaking during a symposium on the British Association report on Social Concern and the Biological Sciences, Mrs Williams, a member of the work-

ing party who conceived the report, said that she had been dependent on a few and sometimes only one scientific adviser. There must be more round-table discussions such as the British Association can provide on controversial biological and medical advances, she said. Parliament badly need greater education and more information. Many of these topics are very suitable one for a free non-party vote but only amongst informed men and women, Mrs Williams concluded.

The working party has been criticised in several quarters for producing a non-report. Countering such criticisms, Professor Bodmer promised that they would now draft concrete proposals and press for these to be put into effect. The working party's life is to be prolonged by a fresh infusion of funds from the Leverhulme Trust, and in its future incarnations will widen its scope to cover other biological advances such as the genetic manipulation of bacteria, but would not venture into thorny paths such as pollution, population and behavioural control. □

Science and politics of molecular biology

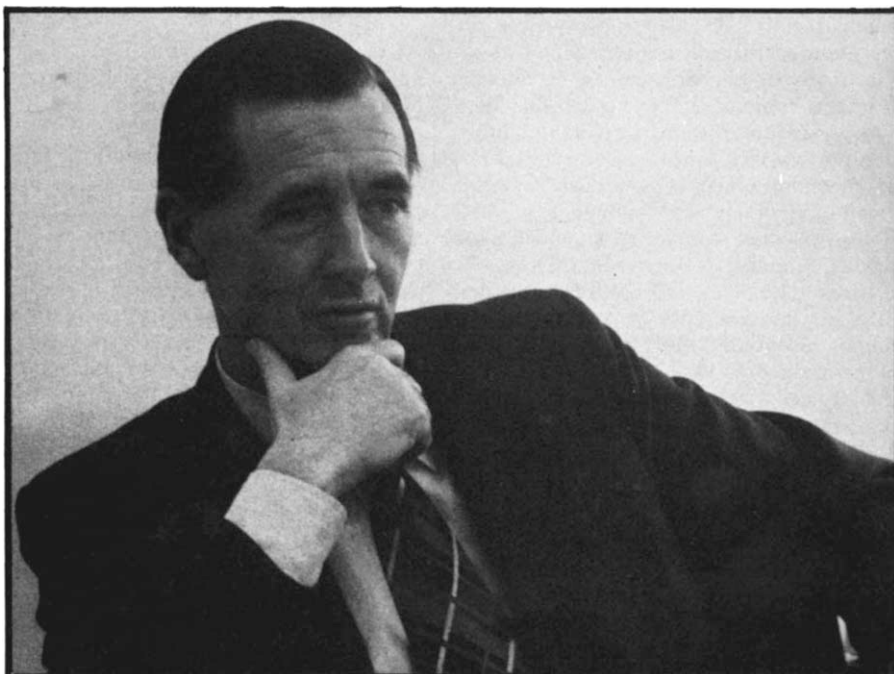
by Eleanor Lawrence

MORE money for molecular biology could be the most cost-effective way of reducing Britain's massive health bill, according to Professor John Paul of the Beatson Institute for Cancer Research. Professor Paul told the British Association Annual Meeting that Britain is now approaching the limits of what our society can pay for in terms of direct medical care, and the only way to eventually reduce the bill was by a three to four-fold increase in government funding for biomedical research and especially for molecular biology.

Ill health costs Britain about one quarter of the annual Gross National Product each year when loss of production and social security benefits are included. But medical research accounted for £800 million, only 0.15% of the total GNP (1972 figures), of which the government contributed £25 million to basic research through the Medical Research Council. Defence research on the other hand was supported to the tune of £250 million in the same year.

In the past, biomedical research had brought the infectious diseases more or less under control, said Professor Paul, and indeed we could not afford such massive spending on ill health now, if this had not happened. But we are now left with a residue of degenerative diseases and the second most frequent cause of death—cancer, which had proved difficult to combat by traditional medical means. Professor Paul believes that we are on the brink of a rapid advance in the understanding and treatment of cancer, and that this will result from the application of the ideas and methods of molecular biology.

Twenty-one years of basic research in molecular biology have brought it to a point at which, through the relatively new branch of 'molecular pathology', its principles and methodology can be applied to the study of disease. About £1 million is spent on molecular biology, of which the MRC contributes the greatest part. Although research into molecular biology takes up about 5% of the MRC budget, in national terms this is very modest financing compared with for instance the United States. Professor Paul believes that molecular biologists should not hesitate to become involved in the 'political' arena and argue on political and social grounds for an increase in government finance as it is becoming increasingly clear that molecular biology ("that apparently most abstract of biological sciences") is a highly political subject. □



Room at the top in space

A YEAR ago the ministers of the 10 European Space Research Organisation (ESRO) countries reached final agreement on the formation of a single European space body—the European Space Agency, and a package of projects on which to embark itself. The Agency was due to come into force on April 1 1974, already three months later than the original target date, but in the event this date too proved ill-chosen. The ESA is still not in being.

The lapse is more apparent than real. The money is secure with guarantees from each of the 10 member countries and with what amounts to a bond deposited on the largest project of all—the \$300 million development of the Spacelab element in the American space shuttle programme for the 1980s.

Most of the planned projects are trundling on and fairly well on schedule and within their budgets. A key meeting of national delegates takes place on September 27, and at the full ESRO Council on October 8 there is every hope that 'baptism' of the new management can be confirmed.

It may take another two years for the ESA Convention to be ratified by all 10 member states. In the meantime the organisation continues to run on the ESRO Convention. ESRO is already a very different animal from the ESRO of last year. The total budget this year is \$240 million, against a mere \$40 million in 1973. The Spacelab contract has been let. The more than two years of stalemate on the joint US-ESRO-Canada Aerosat project—to set up a pre-operational system for evaluation of air traffic control and navigation satellites in cooperation with air-lines flying the Atlantic—has been resolved.

This has hinged on what to Europeans has seemed a technicality, finding an acceptable industrial partner among American companies to act for the US Government opposite ESRO and Canada on the management board. The Memorandum of Understanding between the three parties originally to be signed in January 1972 was finally completed in Paris last month. Last week the Aerosat Board chose Comsat (out of five contenders) as the American partner and its head visits Paris soon to pursue details. The target date for launching the first Aerosat over the Atlantic, mid-1978, should now be met.

The credit for most of this must go to the head of Administration, Roy Gibson (pictured above), who has been acting director-general for the past months and was ESRO's chief negotiator at the original Aerosat talks in 1971. Latterly the work of the eight senior posts in ESRO has been shared between three people, with Gibson sustaining the lion's share.

In the face of the increased volume of work this hiatus at the top at ESRO has been serious. Two of the best men among ESRO's senior and most experienced staff have left for good. The five senior posts that are vacant cannot be filled till the director-general is appointed, since political considerations enter into the distribution of seats among the member countries. Of the sitting executives, one is English, one a Swede, one Italian. The most widely promoted candidates for the top job are the French ex-head of ELDO, the much respected General Aubinière, and a German civil servant, Dr Schmidt-Kuster. There is also a case to be made for Gibson himself. □

"TRYING to pour a water-butt through a bootlace" was how one Services officer described pre-satellite long-distance communications for defence. That was in 1969 when Britain first adopted a satellite-borne defence communications system—*Skynet*—for its own exclusive and interference-free use, making Hong Kong and ships at sea (in the Gulf of Carpentaria for example) as instantly accessible as Aldershot from Whitehall. Although the satellite itself, *Skynet I*, was built (to a tight £6 million contract) by Philco-Ford in America, the hard-headed specification was British and the ground-based terminals to communicate with it were British-designed and built. The *Skynet* system must rank as the most enterprising and successful British space venture yet achieved with several world 'firsts' to its credit. It was the first operational defence system to use a geostationary satellite; and, the 3½-foot shipboard terminal, *Scot*, remains the smallest and most ingenious 'earth-station' for satellite communications yet developed, and the envy of the US Navy.

The original *Skynet* satellite (*Skynet I*) has now exceeded its planned operational life and is due for replacement. To ensure continuous operation in all circumstances the original satellite, poised over the Indian Ocean, was duplicated. The second could act as standby if the first developed faults; otherwise it could provide additional capacity in an emergency. In the event only one of the *Skynet I* series ever became operational. In its companion the solid fuel packing of the centrally situated apogee motor had an undetected crack which caused the motor to explode on ignition, effectively disposing of the satellite at the same time. It seems that the single *Skynet I* in orbit managed to sustain its role without a standby.

Plugging the hole in Skynet

from Angela Croome



Skynet: the ship-borne end

Last January the first of two replacements was launched by Thor-Delta from Cape Canaveral. It too came to grief so now there is the prospect of the second, *Skynet II*, phase of the system, also operating with a single satellite despite 100% planned redundancy. On this occasion the second stage of the launch rocket malfunctioned in the sense that the jets of the motor jammed hard over while firing, causing the vehicle to corkscrew wildly and subjecting the satellite to forces greatly exceeding those allowed for in the design specification. The satellite was 'missing presumed lost' for three days but was then picked up by a United States Air Force tracking station over the Pacific. It was still functional and responding to interrogation and commands but in a hopelessly low orbit. An attempt to 'kick' the satellite out to an orbit clear of the Earth's atmosphere with the onboard apogee motor failed. Nonetheless the

British contractors, Marconi Space and Defence Systems Ltd, were well pleased with how well the spacecraft had stood up to its very rough passage.

In the last week of August the standby *Skynet II* was dispatched to the United States in readiness for launch in November to take over Britain's overseas defence communications role for a further three years. The overall design and engineering concept of *Skynet II* is similar to *Skynet I*; it may be considered a 'stretched' *Skynet I*. It is about a third larger than its predecessor, however, and has a considerably greater communications capacity. The ease, convenience and immediacy of 'voice-contact-by-satellite' has, not surprisingly, proved popular with the Ministry of Defence, as transatlantic businessmen similarly welcomed the coming of Intelsat.

Demand for faster telecommunications grows with supply. This has become a truism in the world of commercial telecommunications. Predictions of the growth of phone traffic and especially overseas phone traffic are always too low. No doubt the same can be said of defence communications. It is not difficult to imagine the asset that *Skynet* must have been and still is to the British bases in Cyprus during the recent emergency. Yet the future of *Skynet* is hazy. No decision has yet been made on a backup for *Skynet II*, although the end of its planned life is not so far off. Defence planning—where a new weapons system for instance takes ten to twenty years to develop—has long made nonsense of five-yearly changes in Government. Can anything so fundamental to the total defence operation as communications, be left to the shifts of party politics? While Britain continues to have overseas defence commitments, a return to "bootlace" communications would be a false economy.

Life sentence

AN astronomical surprise for dilettantes and professionals alike was provided by Sir Bernard Lovell during a discussion at the BA meeting. He delivered a severe body blow to the popular notion that there may be around 100,000 technological civilisations in our Galaxy.

In the past, Sir Bernard said, one extremely important factor had been omitted from the calculations. Nobody had yet taken account of the fact that at least 90% of the 100,000 million stars in the Galaxy are K or M stars, which exhibit characteristics that could well preclude the development of organic life in their vicinity. For a start, they only exist for about 10 to

100 million years before becoming black dwarf stars.

It is extremely unlikely that these stars could develop a solar system in so short a time, and in any case that hardly seems long enough to allow for the evolution of an advanced civilisation. Which is just as well really, because any organic life which might evolve would find itself in an extremely inhospitable environment.

Sir Bernard explained that K and M stars have extraordinary flare properties: blasts often last for periods of up to several hours, during which the normal intensity of emission doubles. The radiation effects would of course be quite lethal to most complicated life forms. □

● BECAUSE of an error in transmission, *Nature's* account of the recent NAS statement on plasmid engineering contained an inaccuracy which might have caused a misunderstanding about the moratorium. Under the heading of types of research which should be halted, we listed "autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not presently carry such determinants . . ." This should have read: "Construction of new autonomously replicating bacterial plasmids . . ." etc. We regret any inconvenience this error might have caused. □

CANADIAN scientists are pondering the implications of federal government appointments that have followed in the wake of July's general election. A Cabinet shuffle has brought a new minister and secretary (deputy minister) to the Ministry of State for Science and Technology, which celebrates its third birthday this autumn.

Actually, "new" is not quite the word for the man named to head the ministry as successor to the Hon. Jeanne Sauvé, now minister of Environment Canada. C. M. ('Bud') Drury is not only one of the most experienced members of the Trudeau Cabinet, having served for some years as president of the Treasury Board—he was also *de facto* minister of science before such a post existed officially. This responsibility fell to him for a number of reasons: his post with the Treasury Board, his position as the minister to whom the National Research Council of Canada reported, and his chairmanship of the Privy Council committee on scientific and industrial research.

In addition to the science and technology portfolio, Mr Drury has been given Public Works, and the question scientists are asking is, does his dual appointment indicate a downgrading of science in the priorities of the government, or does this appointment of a senior and experienced Cabinet minister indicate an upgrading of the ministry?

In terms of experience, Mrs Sauvé was a junior minister, science and technology being her first ministry appointment. Her move to Environment Canada can only be seen as a promotion, because it is a far larger department.

Her predecessor, Alastair Gillespie, who was Canada's first science minister, was also a new boy, who has since

Canada gets a new science minister

from David Spurgeon, Ottawa



'Bud' Drury: experienced Trudeau man

been promoted to the Ministry of Trade and Commerce. The fate of these first two science ministers confirms the idea that the government has been using the post as a training ground for rookies.

But now the issue is complicated by the rumour that Mr Drury's appointment as science minister is a temporary one. This poses two questions: Who will be the permanent minister? And will the new appointee have more or less political clout than Mr Drury?

Some see Mr Drury's appointment as a holding operation—yet a choice that will assure no reduction of importance accorded science in federal policy, because of his personal experience in the field. Because the ministry is not an operational department, its political influence depends largely on the status of the minister, or the im-

portance attached to the portfolio by the Prime Minister, or both.

The implications of the new deputy minister's appointment are not entirely clear, either. Formerly deputy minister of health, Dr Maurice LeClair is expected by some to exert a more cohesive force on the day-to-day operations of the science ministry than his predecessor, Dr Aurèle Beaulnes.

But some expect his policies to be a good deal less open: Beaulnes was always ready to talk to the press (usually at great length); health ministry officials have been more secretive and restrictive. In fact, Dr LeClair went so far last spring as to issue a directive prohibiting health department scientists from attending a forum on science policy set up by SCITEC, an umbrella organisation that is striving to represent all branches of science and technology in Canada. The given reason was "that this whole area of science policy is at such a ferment at the present and so deeply tied in with basic government policy that it would be inappropriate for our scientists to be in attendance at the SCITEC meeting."

Later, on the day of the conference, after a public service union pointed out to the department that the directive abrogated personal freedom, and that a scientist was already bound by an oath of confidence to the government, the department modified its stand: its scientists could attend as long as they did so as individuals and not as representatives of the department.

Both Drs LeClair and Beaulnes have medical backgrounds, which is a cause for concern among some scientists who feel this key post should be assigned to someone whose training and experience better equips him to understand research in all fields.

First Netherlands satellite in orbit

THE first Netherlands scientific satellite ANS was launched from the Western Test Range in California by NASA on August 30, 1974, by a four stage solid fuel Scout rocket serving as launch vehicle.

The Astronomical Netherlands Satellite carries three scientific experiments for astrophysical observations and comprises instruments for the measurements and classification of stellar UV radiation and X-ray sources in space.

The spacecraft's systems are in good condition and the attitude acquisition phase during which one side of the satellite will be locked to the sun, is

running as planned. However the rotation around the Earth differs from the original planned near-circular orbit of about 500 km altitude as present orbit has an apogee of 1150 km and a perigee of 260 km.

In the coming days all systems of the satellite will be checked and calibrated before measurements are initiated. In spite of the deviation in altitude it is expected that by reallocating and adapting the observation programme the planned investigations can be executed according to schedule and will last for at least half-a-year before leaving the sun-lit area. For the first time complete operation control of an European satellite is performed at ESOC, Darmstadt, in Germany, by a team consisting of Dutch NLR and ICANS members.

Indians' blast

A PUBLIC opinion poll of 250 adult literates in each of the cities Bombay, Calcutta, Delhi and Madras shows how strongly the Indian public feel about the recent nuclear blast. The questionnaire conducted by the Indian Institute of Public Opinion reveals that 89% of the sample believe that the test has raised India's prestige in the international community and 80% believe that peaceful nuclear explosions will help faster economic growth through use in mining and other fields. Nearly a half want to supplement the peaceful uses programme with a programme to build nuclear weapons while only a third of those questioned wanted the government to use nuclear energy solely for peaceful purposes.

news and views

Successor to Anger's gamma camera?

DURING the past fifteen years gamma cameras have been introduced into the regular hospital armoury of nuclear medicine. In the hands of a specialist this camera became a diagnostic tool of great importance, particularly for detecting and localising cancer lesions. The generation of γ cameras in current use derives its pedigree from the concept of Hal Anger (*Rev. scient. Instrum.*, **29**, 27; 1956), who used lead collimators to produce an image of the radioisotope distribution in a scintillating crystal viewed by an array of photomultipliers. On page 132 of this issue of *Nature*, Todd, Nightingale and Everett propose a new principle of γ -ray imaging. Using arrays of position-sensitive detectors and selecting multiple interactions it becomes possible to calculate the position of the source of a γ ray from the formula for Compton scattering. In this way the use of collimators is completely avoided. The image formation in the proposed system is different from that in the Anger type of camera. Instead of building up the image from a dot pattern, the Compton γ camera produces families of ellipses with the common point corresponding to the source of radiation.

The method of implementation of the new concept is hardly mentioned in the communication. In fact, the success of the proposed system probably depends in a most crucial way on the nature of the radiation detectors and on efficient, very fast collection of data. Recent developments in the technology of solid state detectors and the availability of very fast mini-computers are making the

proposals a practical possibility, but it is worth noting that to date no commercial γ cameras based on semiconductor detector technology are offered. The Southampton group is therefore attempting to improve the design of the gamma camera in more ways than one.

The main advantages of the proposed new system lie, first, in its ability to collect γ radiation over a larger solid angle than is possible through a system of collimating holes, with a corresponding reduction of the necessary patient dose, and, second, in the possibility of attaining higher positional resolution than the existing cameras. Modern, high performance Anger cameras have resolution indices of about half a centimetre. The authors' estimation of the resolution possible in their system is in the region of 1–2 mm. The ability of detecting smaller malignant lesions is always uppermost in the minds of clinical diagnosticians.

With a suitable fast data collecting system the high potential sensitivity of the Compton γ camera will make it the instrument of choice for fast dynamic studies with radioisotopes, for example, for the study of cardiac output and the blood circulation in limbs. Furthermore, as Todd *et al.* indicate, the computing system associated with the camera can select events originating at a certain depth in the body and can thus yield the tumographic information in any desired place.

It remains to be seen if the Southampton group, after presenting a new and exciting idea, will be able to break through the technological barrier and produce a working model of the camera that will make it possible to evaluate all the predictions and expectations of its performance.

K. V. ETTINGER
D. A. CAUSER

What makes a molecule odorous?

THE olfactory quality of a molecule has always proved notoriously hard to predict, but another attempt has recently appeared (Schiffman, *Science*, **185**, 112; 1974). This work is mathematical in nature and applies multidimensional scaling to known data.

How much progress has been made since the confrontation between Amoore and Wright in the pages of *Nature* a few years ago (**233**, 231, 270; 1971 and **239**, 226; 1972)? In particular, can one yet distinguish between the theories of Amoore, Wright and Davies, preferably by a clear refutation of two of these theories?

The answers to these questions are complicated by the modifications that all three theories have undergone in recent years, and it seems also that the number of olfactory theories is growing, rather than diminishing. (A full account of the principal theories and results up to 1970 may be found in *Handbook of Sensory Physiology*, **4**, (Part 1, Olfaction), Springer-Verlag, 1971).

Schiffman uses measures of psychological similarity as the input for her multidimensional scaling: if two substances are judged by human subjects to have quite similar odour qualities, these two substances are placed near each other in multidimensional quality space. Of course this is not new in itself; but what is new is that the known similarity data are now re-analysed by a general non-metric

multidimensional technique, which shows that only two dimensions are required to account for the known similarity relationships between many different compounds.

What new information emerges from such two-dimensional odour maps? First, it seems that there are no sharply defined areas which would correspond to possible 'primary odours'—the odour types all merge into one another, for example, from floral and fruity areas to areas of resinous (for example, turpentine-like) odours. Or, in Schiffman's words: "There are no clear psychological groups or classes of stimuli, merely trends". Unfortunately, she does not present any data on 'musky' compounds, usually regarded as constituting a well-defined group.

Second, can these psychological similarities be related to the usual molecular properties? Schiffman claims that Amoore's original (1952) theory that molecular shape

This is the last News and Views prepared by Sally Bunney who after nearly seven years with *Nature* is off to a post as medical librarian in Oman. She goes with the best wishes and affection not only of the staff but also of an army of correspondents. Mrs Gillian Boucher takes over Miss Bunney's position.

uniquely determines olfactory quality is not confirmed, but this conclusion was reached some years ago by Amoore himself, who modified his "site" theory to account for trends rather than for sharp "fit" or "not-fit" categories of molecular shapes.

As regards Wright's spectral theory, this gives a good correlation with the odorants in two broad categories (pleasant and unpleasant), but five pleasant odorants are incorrectly classified as unpleasant on the basis of their Raman spectra.

As regards the "penetration and puncturing" theory of Davies, in which the lipid part of the plasma membrane is held to be responsible for olfactory effects, Schiffman reports that lipid solubility may be necessary for olfactory stimulation to occur (since all the odorants were soluble in ether and there was no trend of odour type as a function of water solubility). There is apparently no evidence against the two-dimensional odour map earlier published by Davies.

So no single physico-chemical variable can account for odour qualities. But how many such variables are required? Schiffman used suitability weighted functions of Raman intensity, molecular weight, number of double bonds, functional group and cyclic structure, these being taken as simultaneous but separate variables which, empirically weighted in combination, are used to correlate molecular properties with the psychological similarity map. Altogether 25 weighting factors are chosen to give the best fit. The final correlation coefficient is 0.76, which in these circum-

stances is distinctly disappointing: as Wright has pointed out, Amoore's odour similarity values (admittedly over a limited range of odour) have a correlation coefficient of 0.85 with the square root of the molecular weight. Furthermore, as Mazziotti has shown (*Nature*, **250**, 645; 1974), there is even quite a fair correlation between odour type and boiling points, particularly within groups of substances whose molecules have the same functional group. Perhaps Schiffman's most important contribution to the debate is that molecular shape as such is not said to be significant.

Future progress in the field of odour must surely be concerned with mechanism of the initiation of the nervous impulses in the olfactory epithelium. Adrian, many years ago, found that different regions of this epithelium in the rabbit and the hedgehog are relatively selective to stimulation by certain types of odorant molecules. Since the lipids may well be responsible for the initial adsorption of the odorants from the air stream, and since the lipid structure may then be locally disorganised by the odorant molecules, one predicts differing properties of the lipids in the various regions of the olfactory epithelium.

This urgently requires testing experimentally by an analysis of lipids taken from the various regions of the epithelium. Only by experimental tests of such physical theories is there likely to be real progress in the understanding of olfaction.

J. T. DAVIES

Clonal selection hypothesis under fire

BIOLOGICAL research in recent years has acquired various dogmas, the most famous of which is probably the central dogma of the molecular biologists. Immunologists, not to be outdone, have espoused the clonal selection hypothesis as their central concept to provide a framework for the understanding of classical antibody synthesis. A recent statement of this notion says: "Antibody molecules consist of heavy and light polypeptide chains, the variable sequences of the 110 N-Terminal amino acid residues of these chains determine antibody specificities; lymphocytes are cells that express one pair of structural genes for these sequences; *one lymphocyte and all of its descendants make identical antibody molecules* (my italics); these are placed as receptors for antigen on the outer lymphocyte membrane; the attachment of antigen to the combining sites of these receptors is a step in lymphocyte stimulation leading to cell proliferation, antibody secretion, and thus to an amplification of this selected antibody" (second *Annual Report of the Basle Institute for Immunology*, page 1).

Although several of the items of this concept thus stated could be questioned, the present interest is in the italicised phrase which seems to be contradicted in part by a recent communication by Cunningham and Fordham (*Nature*, **250**, 669-671; 1974). These investigators present evidence that sometimes single antigen stimulated cells can engender clones of cells of which not all the members of the clone are producing the same kind of antibody.

Mouse spleen cells were cultured *in vitro* in the presence of bacterial lipopolysaccharide and sheep red blood cells. After 24 h the cells were harvested and dispersed in microdroplets under oil in fresh medium with a mixture of red cells from two standard sheep and complement. In these circumstances the antibody from some of the cells diffuse into the surrounding medium and, in the presence of complement, soon lyses the sheep red cells giving rise to a more or less clear plaque around the antibody-producing cell. The plaque-forming cells were isolated by micromanipulation from the microdroplets and seeded on to a feeder layer of irradiated mouse spleen cells and sheep red cells

were again added. After about 2 days some of the isolated plaque-forming cells had divided. Their progeny cells were re-isolated where necessary in the presence of trypsin to disaggregate them and they were added individually to a mixture of fresh medium, complement and red cells from the two standard sheep. These mixtures were pipetted into Cunningham slide chambers (Cunningham and Szenberg, *Immunology*, **14**, 599; 1968), and the resulting (19S) plaques were characterised as either clear, partial or 'sombbrero'. Clear plaques are supposed by the authors to arise around cells which have produced an antibody (or antibodies) which recognises antigens common to both the target red cells. Partial plaques have some unlysed red cells and (despite the lack of a morphological criterion of distinction) these are interpreted as arising around cells which produce antibody capable of binding effectively to only one of the kinds of red cell. Sombbreros are supposed to arise around cells which are producing antibodies for common specificities but with higher avidity for one of the kinds of red cell than the other. About 10% of all isolated cells formed clones and of these about 10% were found to give rise to heterogeneous plaques. Most of the heterogeneous clones had only two kinds of plaque forming cell. Two had three kinds of cell and one clone (of twelve cells) gave rise to four clear plaques, five sombreros and two partials. One could not be diagnosed.

On the basis of these results the authors postulate that, contrary to the precepts of the clonal selection theory, diversity in relation to antibody-producing capacity can commonly (if not always) arise after contact with antigen rather than be pre-existent. The approach is ingenious and owes much to the use of the polyacrylamide gel micro-culture plates developed by Marbrook and Haskill (*Cell. Immunol.*, in the press). Whether the evidence presented by Cunningham and Fordham will withstand the barrage of criticism which will be put up by the immunological establishment remains to be seen but it is healthy that after such a long run unopposed, the Clonal Selection Theory is taking a few knocks.

A. J. S. DAVIES

Can neutron starlight be seen?

AMONG the more exotic inhabitants of our Galaxy are the enigmatic neutron stars. Although these objects are about the same mass as ordinary stars, they are only a few kilometres in diameter, and are therefore unbelievably dense. So much so that their constituent atoms are entirely crushed by gravity into neutrons. Despite having been predicted theoretically for many years, firm evidence for their existence had to await the discovery and interpretation in the late 1960s of the now famous pulsars. Pulsars are radio sources which emit bursts of energy at very regular durations, usually about once a second. Current models of these cosmic clocks assume their regular ticking to be caused by the rapid rotation of an extremely compact object coupled to a magnetic field. The compact object is generally thought by astronomers to be the long awaited neutron star, born during the violent aftermath of a supernova explosion.

Models of the pulsar mechanism are necessarily tentative, but the pulses of radiation are believed to originate not from the neutron star itself, but rather from the surrounding magnetosphere. This then leaves open the question of what radiation might be expected direct from the neutron star surface. The answer to this depends on the temperature of the surface, which is really an unknown factor. General considerations, however, point to this radiation being in the X-ray region, so that the exciting possibility emerges that the nascent science of X-ray astronomy might provide important data for astronomers studying neutron star structure and pulsar mechanisms.

Actually, the idea of searching for X-rays from neutron stars is not new. Before the appearance of pulsar astronomy, it remained the best hope for the eventual discovery of these strange objects. The excitement generated during the past few years by the spectacular pulsars has, however, quite eclipsed the consideration of X-ray detection of neutron stars, and the subject makes a new appearance in the August 9 issue of *Science* (185, 487-490; 1974). The resurrection is promoted by two American astronomers, Greenstein of Amherst College Massachusetts, and McClintock of MIT. These authors point out that this X radiation bears the same relation to neutron stars as continuum starlight does to ordinary stars. And where would astronomy be without starlight?

Recently, strenuous efforts have been made to construct theoretical models of neutron stars. The problems involved are formidable, for in almost every

respect these objects represent matter in an extreme condition. Indeed, the entire neutron star may be envisaged as a sort of gigantic atomic nucleus. A correct understanding of such a strange object requires advances in several separate branches of physics. Consequently, a direct observation of neutron star radiation would provide the only source of observational support for theories of matter in this high density condition.

Specifically, Greenstein and McClintock suggest five pieces of information which could be deduced from a careful measurement of X-ray spectrum. The spectrum itself would give the surface temperature of the star and hence, knowing the total flux, its radius could then be estimated, thereby confirming (or not) that the source really is a highly compact neutron star. In turn the radius could be used to determine the mass and the pulsar magnetic field, at least approximately. In addition, a knowledge of the magnetic field, temperature and mass enables the age of the neutron star to be calculated, with the possibility of a fruitful comparison with the associated pulsar age which may be deduced from the rate of slowing down of the pulsar period. Finally, it is in principle possible to determine the internal temperature of the star, with a possible cross check here too from the details of the quasi-exponential period decay which has been observed to occur after peculiar period jumps in the radiation pulses.

In their article, Greenstein and McClintock outline three theoretical procedures which may be used to obtain an estimate of the temperatures of neutron stars. The first is simply to try to calculate the rate of cooling as from the time of formation. The second is to examine various dissipative processes which generate heat inside the object as it rotates. In both these methods the condition of the relevant theoretical models does not inspire much confidence in the result, so that a crude upper limit may be placed on the temperature by summing the total available energy. Turning to the observational aspects of the subject, the authors paint a rather pessimistic picture. There is no observational evidence at all yet for thermal X rays from neutron stars. The best potential candidates are thought to be the middle aged pulsars—perhaps 100,000 years old—whose direct radiation would not be swamped by the pulsar radiation from the magnetosphere. From the present non-detection of X rays from the known pulsars by the Uhuru X-ray observatory, upper limits on the

temperature of 21 nearby pulsars are quoted. A comparison of a sample of these limits with the theoretical values obtained using the three methods described above is presented. Unfortunately, in all cases the theoretical values lie well below the observed upper limits, so that little of value may be gleaned from this.

Despite the rather discouraging situation so far, Greenstein and McClintock argue their case well, and it would be surprising if further observational and theoretical work were not initiated on this difficult but important topic for astronomers. And in view of the fact that the neutron star represents the most extreme density of matter in the observable Universe (greater densities lead to unobservable black hole matter) there is also a general scientific interest in their direct observation.

P. C. W. DAVIES

Checking on laser fusion

from a Correspondent

IN May this year, KMS Fusion Inc. claimed to have produced, for the first time in the United States, thermonuclear neutrons from a laser-compressed deuterated target. (The background to the laser-compression approach to fusion is outlined in *Nature*, 239, 139 and 129; 1972.) K. M. Siegel, chairman of the company, later amplified some of these claims, which are the outcome of a private corporate investment which now amounts to \$17.5 million, in a talk given at Harwell on July 8.

Between May 1 and July 8, about 35 neutron-producing shots on deuterated and D-T targets had been made, of which 80% were successful. The prompt neutron yield (measured with a time resolution of 2 ns) was as high as 5×10^5 when the target chamber was evacuated; the total yield increased to 1.8×10^7 when deuterium gas was admitted to the target chamber. (The latter result duplicated earlier Russian yields, in which neutron time-of-flight measurements were made with a detector resolution of 5 ns over flight paths of more than 3 m.) X-ray pinhole photographs, with a spatial resolution of 5 μm , demonstrated that the implosion and compression of a hollow target was spherically symmetric. Time-resolved X-ray emission measurements suggested that a density increase of 80 times occurred when two opposing Nd-laser beams (total energy < 100 J) were focused by ellipsoidal mirrors on to the (suspended) solid state target.

This compression, and the prompt neutron yield, were said to be consistent with computer predictions.

These claims have unleashed some controversy within the international scientific community. Everyone agrees that 'scientific break-even', in which the energy of the neutron output is roughly equal to that of the incident laser pulse, would produce so many neutrons ($\sim 10^{14}$) that a detailed and unambiguous determination of their spectrum and physical origin would be possible. Severe problems arise, however, with neutron diagnostics at the present level where, relatively speaking, only a handful of neutrons (10^{-8} of the break-even yield) are available for examination. Additional diagnostic techniques which can provide independent checks on plasma density or temperature are therefore necessary until well documented 'break-even' yields have been achieved.

At densities less than approximately 10^{19} electrons cm^{-3} , measurements of refraction or scattering have often proved a reliable diagnostic. Attwood and Coleman (*Appl. Phys. Lett.*, **24**, 409; 1974) have recently discussed a holographic micro-interferometer, operating at a wavelength of 5320 Å, which measures the optical path length through plasmas at densities exceeding 10^{20} electrons cm^{-3} . The duration of their laser pulse 10^{-10} s limits the spatial resolution to 30 μm for plasmas moving with velocities less than 3×10^7 cm s^{-1} . Shorter laser wavelengths permit penetration into denser plasmas, and reduce beam deviations caused by refraction at strong density gradients. (Thus, the United Kingdom Culham Laboratory is using a 3471-Å holographic interferometer for density measurements with a resolution of 10^{-9} s on sub-kilojoule CO_2 laser-filling experiments.) For penetration to densities of interest in the context of laser fusion ($10^{25} \sim 10^{27}$ electrons cm^{-3}) sources of high spectral brightness with wavelengths of less than 10 Å will be needed; there is therefore a considerable incentive to develop coherent X-ray sources for refraction and absorption measurements in this regime.

Temperature measurements are also difficult (see Puell *et al.*, *Z. Naturforsch.*, **25a**, 1815; 1970) and at high incident intensities the electron velocity distribution may not be completely Maxwellian. For example, at Los Alamos, McCall *et al.* (*Appl. Phys. Lett.*, **25**, 108; 1974) have demonstrated a significant departure from equilibrium when a thin polythene (slab) target is irradiated at incident intensities of 10^{16} W cm^{-2} ($\lambda = 1.06 \mu\text{m}$) or 10^{14} W cm^{-2} ($\lambda = 10.6 \mu\text{m}$). For these measurements a six-channel Bragg crystal spectrometer was used to analyse time-resolved X-ray brems-

strahlung between 4 and 50 KeV with an energy resolution of some 4–12%. Conventional scintillator/photomultiplier detectors were used in this work; but for faster detection Key, working with the Garching group, has recently shown that sub-nanosecond X-ray vacuum photodiode detection should be possible. Peacock and colleagues have exploited the rich line spectrum emitted from partially ionised $(\text{CH}_2)_n$ plasmas; a grazing incidence spectrograph, intensely calibrated over the range 5–45 Å (*J. Phys. E*, **6**, 854; 1973) is used to unfold line intensities and recombination continua. Considerable care is required to achieve reproducible results with such photographic techniques.

A wide range of experiments has been reported from laboratories in France, Germany, Japan and the Soviet Union, in which the intensity and spectrum of light back or side-scattered from the target at frequencies near the fundamental laser frequency and its harmonics have been measured; such data should provide valuable information concerning nonlinear absorption mechanisms which are of importance to the concept of laser fusion. Within the next decade compression experiments capable of delivering some 1 to 10 kJ of laser energy on to carefully designed targets are planned at KMS, the University of Rochester, Lawrence Livermore Laboratory and Los Alamos in the United States, the Lebedev Institute in the Soviet Union, and probably at other national laboratories as well. The outcome of these experiments will be crucial, since the demonstration of a break-even yield of neutrons would constitute a definitive milestone in the history of thermonuclear research.

A new role for the glial cell?

from a Correspondent

GLIAL cells are derived embryologically from the same group of ectodermal cells that gives rise to neurones and they were distinguished from nerve cells more than a century ago by histologists. About half the volume of the vertebrate brain is composed of glial cells and these cells outnumber neurones by a factor of roughly 10 to 1. But although glial cells can be studied by morphologists, physiologists know very little about their function. It is usually argued that because nerve cells are closely enveloped by glial cells the latter provide a 'supportive function' but just what kind of support has not been clear.

A clue that one aspect of this support might be to provide a protein

vital for the growth and differentiation of the neurone can be found in the discovery, reported by Longo and Penhoet (*Proc. natn. Acad. Sci., U.S.A.*, **71**, 2347; 1974), that a tumour derived from glial cells contains the protein known as nerve growth factor (NGF). The protein was obtained from a rat glioma tumour by the method used to purify NGF from mouse salivary glands. It cross reacted with antiserum to mouse NGF, gave similar responses to NGF in two biological assay systems and three of the proteins in the tumour extract had the same isoelectric points as the three fractions of mouse NGF. The tumour extract, however, also contained other proteins, as was shown by electrophoresis and bioassay.

Nerve growth factor is a basic protein (or class of proteins) which stimulates the growth of embryonic sensory ganglia and whose presence is required for the normal growth and differentiation of peripheral sympathetic noradrenergic neurones. The fact that high concentrations of NGF are only found in the submaxillary gland of the mouse and, even there, only after puberty, has long been a puzzle. Because animals are uniquely sensitive to the neutralisation of NGF by antiserum during the perinatal period, it has been argued that there must be an important unidentified source of NGF in the newborn animal. Longo and Penhoet now suggest that this source may be in the glial cells: they put forward the hypothesis that "glia synthesise and secrete NGF which then may act as a 'pleiotypic' stimulant for the neurone".

One of the properties of glial cells—their supportive role during regeneration of damaged nerve axons—is consistent with this hypothesis. Noradrenergic neurones in the brain display a remarkable ability to regenerate after damage to their axons. Studies in Sweden have shown that NGF injected into the cerebrospinal fluid or directly into the brain close to the locus coeruleus (the site of origin of a major group of central noradrenergic neurones) stimulates this regenerative process (Stenevi *et al.*, *Brain Res.*, **69**, 217; 1974). This team has also found that intracerebral administration to rats of antiserum to NGF inhibits the regenerative growth of central noradrenergic fibres (Bjerre *et al.*, *Brain Res.*, **74**, 1; 1974). Since it is believed that NGF does not readily enter the brain from the blood stream, the NGF required for the regenerative process must originate in the brain. Could the ubiquitous glial cell be the source of NGF in the central nervous system?

It is to be hoped that the observations of Longo and Penhoet on the presence of NGF in a glioma will lead to attempts to demonstrate NGF in

normal glial cells, possibly by the use of immunofluorescence histochemistry. If these attempts are successful and if it is possible to demonstrate secretion of NGF from glia, then the glial cells will be recognised as a kind of endocrine tissue that is wrapped around its target cells, the neurones.

Oppression or altruism in insect unions?

from our Insect Physiology Correspondent

THERE are differences of opinion among students of bee societies as to the origin and evolution of the worker caste. Since the views of Hamilton were put forward in the 1960s, most authors have emphasised the idea of 'kin selection' favouring altruistic social behaviour: the workers (the haploid partheno-genetic daughters of the queen) can promote the persistence of genes like their own more effectively by helping their mother to produce more siblings than by leaving home and mating on their own account. But some authors favour theories of individual selection. Either selection of mothers which can control their offspring, keep them in the nest and suppress their reproductivity; or selection of individuals, which, by their social characteristics of mutual tolerance and continued residence in the nest can benefit both themselves and their mother.

Michener and Brothers (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 671; 1974; *J. comp. Physiol.*, **90**, 129; 1974) have now approached this problem by studying a small eusocial halictid bee *Lasioglossum zephyrum* which burrows in earthen banks. Closely related (within the same genus) to solitary species, this bee is about as low in social level as any known eusocial insect; the average membership of a colony is only 14 bees. As in other eusocial Hymenoptera, the males play no part in colonial life. A large number of experimental colonies, maintained by Lubbock's classic technique, in earth between glass plates, were closely observed and the behaviour of marked individuals was compared and quantified statistically. The queens are indistinguishable externally; although they have the largest ovaries, this is only the extreme of a continuum of ovarian size among the workers; the queens are best identified by their behaviour. If the queen is removed her place is quickly taken by one of the workers.

In her behaviour the queen is characterised by maximum general activity and work on the burrows, a maximum tendency to 'nudge' and disturb other members of the colony and thus to suppress their ovaries; and

a maximum tendency (after nudging a bee) to back down the burrow and draw the nudged bee down into the depths of the nest where it will become occupied in constructing and provisioning. Queens were also observed to eat the occasional eggs laid by workers. Conversely the queen shows minimum activity in pollen collecting, and in guarding the nest; as well as in being nudged, or being induced to follow a backing worker.

All this looks far more like oppression of workers by the queen rather than simple altruism by the workers; it suggests that queens have evolved to control their workers rather than that workers have evolved as altruists to help their queens. Michener is therefore led to believe that control or 'oppression' by the queen has been of fundamental importance in the most primitive eusocial forms. But, as he points out, worker altruism is a necessary consequence of eusocial systems and altruistic behaviour may well be evident sometimes even in the absence of control by the queen.

Lassa fever and public health

from Arie J. Zuckerman

LASSA fever was first recognised in 1969 and four epidemics have occurred in West Africa since then (*Nature*, **241**, 91; 1973). The severity of the illness is emphasised by a high case fatality ratio of 36–67% among patients admitted to hospital, person-to-person transmission, particularly in the hospital environment, and a high risk of infection to medical personnel. To date, 20 medical workers have contracted Lassa fever and nine have died (Monath, *WHO Chronicle*, **28**, 212; 1974). In Africa, the virus was introduced into hospitals by African patients admitted with febrile illnesses which often mimic typhoid fever, malaria or yellow fever. Secondary infections occurred among hospital staff and patients, but it seems that although the primary index cases were highly infectious, the secondary cases, for reasons that are not clear, were not.

Lassa virus is a member of the arenavirus genus (invariably misspelt arenavirus). The virus is related both morphologically and antigenically to lymphocytic choriomeningitis virus and the viruses of the Tacaribe complex (haemorrhagic fever viruses of South America). A number of the related arenaviruses have been isolated from wild rodents and bats, and although it was postulated that Lassa fever is also a zoonosis, confirmation was not obtained until last month by Monath and colleagues (*Science*, **185**, 263; 1974). During the investigation of the epidemic of Lassa fever in Sierra Leone in

1972 (*Morbidity and Mortality Weekly Report*, **21**, 386; 1972) 641 small vertebrates were trapped. Samples of organs and tissues were frozen in liquid nitrogen and sent for study to the Maximum Security Laboratory, Center for Disease Control, Atlanta, Georgia, at present the only laboratory equipped to attempt isolation of this virus safely. Lassa virus was isolated from a single murine species, the multimammate rat *Mastomys natalensis*, which is a common commensal rodent in West Africa adapted to life both within houses and in the fields. It is of interest that rodents of other species commonly found in the area of the epidemic in Sierra Leone (*Mus musculus* and *Rattus rattus*) were not infected, and it seems that these species either resist infection or avoid contact with infected *Mastomys*.

The mode of transmission of the virus from rodent to man or from man to man is not known yet. Similarly, the pathogenesis of Lassa virus infection in its natural host remains unknown. It is, however, reasonable to expect that perhaps like a number of other arenaviruses, which induce a chronic virus carrier state in their natural hosts, for example lymphocytic choriomeningitis, a persistent infection with Lassa virus develops and both horizontal and vertical transmissions occur. Rodents may excrete the virus in urine and saliva and may thus contaminate food, water or the air. Monath *et al.* suggest that the low level of sanitation, the storage of grain and food within houses and the ease with which rodents infest mud and thatch houses enhance the contact between rodents and man. The means by which the virus is spread from person to person is not clear. Medical attendants or relatives providing direct personal care are most likely to contract the infection. Accidental inoculation with a sharp instrument or contact with blood has accounted for a few cases (see *Nature*, **241**, 91; 1973). Lassa virus has been isolated from the pharynx and urine of patients so that indirect airborne spread of the virus as well as mechanical transmission are most likely.

Serological and epidemiological investigations have shown that Lassa fever seems to be limited to West Africa, and there is evidence that the virus was present in the Republic of Guinea in 1952; within the last two years it reached Senegal. Mild and perhaps subclinical infections are now recognised to be not uncommon outside the hospital environment. In the absence of a vaccine, available methods of control of community outbreaks are rodent control and quarantine. It has been pointed out previously, however, that the epidemiological propensities of Lassa fever may be very wide as a

result of fast air travel. Indeed, a number of cases have been treated in a London hospital. Monath (*WHO Chronicle*, 28, 212; 1974) wrote that a programme of surveillance must be instituted both in West Africa and in the developed countries. To this one should add the provision of hospital isolation and laboratory facilities in selected centres.

Sicily as part of Africa

from Peter J. Smith

ACCORDING to Barberi *et al.* (*J. geophys. Res.*, 78, 5221; 1973), the southern Tyrrhenian Sea contains an island arc structure which includes a sediment filled trench along the Ionian coast of Calabria, a metamorphic belt stretching along Calabria into Sicily, a calc-alkaline volcanic arc in an advanced state of evolution (Eolian Islands) and a back-arc marginal basin (Tyrrhenian Abyssal Plain). In short, the Tyrrhenian Sea area is readily interpreted in the light of the destructive boundary between the African and European plates. But where precisely does the plate boundary lie in this region? McKenzie (*Geophys. J.*, 30, 109; 1972) and several other workers have proposed that the boundary line actually crosses northern Sicily, in which case most of Sicily will be a part of the African plate. New evidence in favour of this view has now come from two quite different sources.

Evidence adduced by Barberi *et al.* (*Earth planet. Sci. Lett.*, 22, 123; 1974) comes from the distribution, age, nature and palaeomagnetism of volcanic rocks in the east of the island, although the volcanics are only one of five principal provinces in Sicily. In the extreme south-east lies the Ragusa-Iblei carbonate platform which is not affected by Alpine folding and seems to be an extension of the Sahara platform. In other words, the southernmost part of Sicily is apparently linked geologically with Africa—a view supported by the existence of 20 km-thick continental type crust to the south of the island. Central and western Sicily comprise a Mio-Pliocene resedimentation basin; and in the north there is a series of flysch nappes ranging in age from Cretaceous to Miocene. But the extreme north-east of the island is a crystalline massif forming the continuation of the Calabrian metamorphic belt. In other words, the northernmost part of Sicily is apparently linked geologically with Europe. The view that the Euro-African plate boundary crosses Sicily is thus supported in general terms by the geology.

For a more quantitative picture of

Sicily's behaviour, however, it is necessary to look at the eastern part, which has been a site of dominantly basaltic volcanism since the middle Triassic (although only Cretaceous and younger rocks are exposed). The most recent episodes of (subaerial) volcanism are represented by Mt Etna and Mt Iblei; but all volcanic activity before this was submarine, giving rise to pillow lavas and hyaloclastics emitted through fissures in a carbonate platform. The oldest rocks examined palaeomagnetically by Barberi and his colleagues were Upper Cretaceous dykes (with potassium-argon ages in the range 71–84 million years) cutting hyaloclastics of three different Cretaceous outcrops. From Cretaceous to Upper Miocene no basaltic volcanism occurred in the region, although important activity has occurred since, principally in the vicinity of Iblei and Etna. The second set of rocks studied was therefore of Iblei volcanics within the age range 1.7–5.4 million years.

The palaeomagnetic results are simply stated. The Upper Cretaceous pole is consistent with that for Africa obtained by Shazly and Krs (*Geol. Rdsch.*, 62, 212; 1973) but significantly different from the mean Cretaceous pole for Europe. The Plio-Pleistocene pole for Sicily is consistent with the mean Upper Tertiary-Quaternary poles for the Central European-Russian platform and for the French Massif Central lavas and with the Quaternary poles for Africa and Spain (Gerona volcanics). It thus seems that there has been no significant motion of eastern Sicily with respect to Africa since the Cretaceous. On the other hand, Sicily has moved with respect to Europe since the Cretaceous, but not since the end of the Miocene. The implication, therefore, is that (with the probable exception of the extreme northeast of the island) Sicily has been part of the African plate since the Cretaceous—and thus in all likelihood since at least the Triassic.

Barberi and his colleagues then visualise Sicily's fitting into the evolutionary pattern of the region in the following way. From the Upper Triassic to the Upper Cretaceous the African plate moved south-easterly with a sinistral shear movement with respect to Europe, resulting in a separation of the African and European plates and the formation of oceanic crust. The northern edge of the African plate (and thus Sicily) then underwent distension—a process consistent with the alkalic products of volcanism observed in Sicily for the relevant period. From the Upper Cretaceous to the present the motion of Africa with respect to Europe has been mainly compressional with minor dextral movement—a change also reflected in Sicilian volcan-

ism. Basaltic distensive volcanism was absent from eastern Sicily from the Upper Cretaceous to the Upper Miocene. The occurrence of Upper Oligocene-Lower Miocene andesitic volcanism suggests, however, that the compression between Africa and Europe was accompanied by subduction of the oceanic crust previously formed between Sicily and Europe, a process which gave rise to island arc type volcanism towards the end of the Oligocene. Crustal consumption lasted until the Lower Miocene, when the continental collision occurred; and since the late Miocene Sicily has been joined to Europe, as the Iblei palaeomagnetic data indicate.

An obvious problem with this scheme is the post Miocene and current basaltic distensive volcanism in eastern Sicily, which is difficult to explain in terms of the general tectonic framework of compression. The explanation put forward by Barberi *et al.* is that continental collision has occurred at different points along the Sicilian-Calabrian segment of the plate boundary at different times, possibly because of the irregular shapes of the encroaching continents. For example, the subduction of oceanic crust beneath the Calabrian arc is still active (hence the Eolian island arc), although the continental nature of the crust on both sides of the boundary suggests that crustal consumption is now nearly complete. In any event, since the Lower Miocene eastern Sicily has been the border zone between colliding continental blocks and the oceanic lithosphere subducting beneath the Calabrian arc, and has thus probably been affected by local distension, notwithstanding the general overall compression.

The second piece of evidence supporting the view of Sicily as part of Africa comes from laser geodimeter measurements between the island and Calabria on the Italian mainland. Caputo *et al.* (*Geophys. J.*, 38, 1; 1974) report six sets of such measurements made between September 1970 and September 1973. Needless to say, even with lasers it is a difficult task to determine the very small distance changes likely to be involved over such a short period; and the variations in the lengths of the sides of the Sicily-Calabria geometric net are indeed found to be generally smaller than the measurement errors and data corrections. Nevertheless, Caputo and his colleagues tentatively conclude that a statistically significant displacement between Sicily and Calabria took place between September 1970 and June 1971. During this period (but not subsequently) Sicily was displaced roughly northward with respect to the mainland, implying that it participates in the movements of Africa rather than Europe.

Harmony of the spheres

from Robert W. Cahn

VERY gingerly, the proponents of fibre reinforcement are beginning to approach the serious possibility of using fibre-reinforced materials for service at high temperatures—for instance, in gas turbine components. One central difficulty about this is that the metallic matrix may chemically attack the fibres; for example, tungsten fibres interact chemically in a complicated way with a nickel matrix. The other tricky problem is to keep the shapes of the fibres inviolate. It is not too difficult to generate a regularly spaced array of long, thin fibres in a suitable matrix; much research has been done on the directional growth of monocrystals of appropriate binary and ternary alloys, consisting of aligned fibres (or plates) in a matrix. The trouble is that a long thin cylindrical fibre is not in geometrical equilibrium with its matrix.

In a slowly grown eutectic, the total quantity of fibrous phase will be in equilibrium with the total quantity of matrix: the disequilibrium is purely a matter of geometry. This has long been known to metallurgists familiar with ordinary mild steel. The pearlite (Fe+Fe₃C) eutectoid lamellae, if sufficiently heated, turn into arrays of fine Fe₃C spheres. The process, spheroidisation, is driven purely by the tendency to minimise the total area of Fe/Fe₃C interface. The same can happen to a long fibre in a matrix.

As so often in physical science, one of the key ideas comes from Lord Rayleigh. In 1878 he showed that a cylindrical jet of water tends to instability: it is apt to pinch off at intervals of about eight diameters. Recently, McLean of the National Physical Laboratory, showed (*Phil. Mag.*, **27**, 1253; 1973) that, correspondingly, cylindrical inclusions of lead in aluminium split into separate short cylinders if the aspect ratio (length/diameter) exceeds 8. Each cylinder then gathers up into a sphere. The relevance to the stability of a population of fibres is manifest. Although aluminium-lead is no candidate for high temperature use, it is a useful model system.

With the collaboration of Loveday, McLean (*J. Mater. Sci.*, **9**, 1104; 1974) has now taken his investigation of Al/

Pb alloys a long step forward. The main improvement is one of technique: instead of using X-ray microradiography between anneals, the investigators examined their foils in a 1-MeV electron microscope fitted with a heating stage. With the aid of cinematography, and exploiting the high penetrating power of the energetic electron beam, the investigators were able to trace in detail the stages of spheroidisation and make accurate kinetic measurements. In addition to the advantages of *in situ* micrography, they also achieved a much higher resolution by this technique.

The lead inclusions in the Al+5wt% Pb alloy were turned into cylinders by mechanical swaging (there is no suitable eutectic) and foils cut from this material were heated in the microscope to above the melting temperature of the lead. The spheroidisation process is governed by diffusion of matrix atoms through the cylinders; since the lead was molten diffusion was rapid and so was the spheroidisation. The cylinders either broke up into shorter ones which then turned into spheres, or short cylinders spheroidised directly; Rayleigh's criterion was approximately obeyed. The detailed observations allowed the diffusivity of aluminium in molten lead to be determined for a range of temperatures.

McLean and Loveday were also able to examine the merging of individual spheres once they had formed. The lead spheres behaved just like the bubbles of helium in nuclear fuels which have been extensively investigated in recent years, migrating under the driving force merely of a temperature gradient. The kinetics of merging of large bubbles (diameter $\sim 1 \mu\text{m}$) was studied and found, again, to be controlled by volume diffusion of aluminium in liquid lead. These large bubbles moved at a speed, independent of their size, which was consistent with the estimated temperature gradient in the microscope hot stage. Bubbles with diameter less than $0.8 \mu\text{m}$ moved more slowly the smaller the bubble; this is proof positive that the motion of these small bubbles was controlled by interface energy and not by volume diffusion. Very small bubbles, less than $0.12 \mu\text{m}$ in diameter, did not move at all. This immobility is a new phenomenon and its origin is not clear.

The observations showed the merging of pairs of large bubbles when one catches up another, and the kinetics of this process were consistent with the theory developed by earlier investigators of bubbles in nuclear fuels. It seems that bubbles behave in the same way irrespective of whether they are filled with gas or with molten metal.

McLean and Loveday point out that their observations have practical impli-

cations (quite apart from their relevance to the stability of composites). Free-machining alloys such as leaded brass contain small particles of soft metal in a hard alloy matrix, and it should now be possible to develop a prescription for a combined mechanical/thermal treatment to produce form of spherical dispersion with the best machining characteristics.

Light from QSOs

from a Correspondent

THE QSOs—the quasi-stellar objects, or quasars—are believed by many astronomers to be remote galaxies that radiate extraordinarily large amounts of energy as a result of some violent explosion in the galactic nucleus. In all probability the source of energy is gravitation, but the process whereby some of that energy is converted into visible radiation is ill-understood. Although the total flux may be thousands of times greater than in a normal 'quiet' galaxy, the size of the source region must be quite small, no more than a few light days, since fluctuations in light intensity have been observed over a few days, and with a larger source they would be smeared out. The investigation of possible radiation mechanisms is therefore an important task in contemporary astrophysics, and the suggestions put forward by Colvin (*Astrophys. J.*, **190**, 515; 1974) are of considerable interest.

The popular approach, adopted by nearly all theorists, supposes that the explosive event produces a relatively dense plasma in the radiating volume (perhaps 10^8 or more ionised atoms per cm^3), a strong magnetic field linked to the plasma, and high energy relativistic electrons. These are certainly the ingredients involved in producing radiation from supernova remnants like the Crab nebula, and it is attractive to try using them again with the QSOs. Indeed, the most straightforward hypothesis is that the observed radiation from the QSOs, like that from the Crab nebula, is the synchrotron radiation produced by the spiralling of relativistic electrons round the magnetic field lines. The relativistic electrons act as a link in the chain of energy flow: energy comes to them from the explosive event and is reradiated by them as synchrotron radiation.

Unfortunately, difficulties appear on a closer examination. The observed spectral distribution of the radiation does not conform very well with that expected for synchrotron radiation, and in the physical conditions of a QSO one could expect the relativistic electrons to lose energy more readily through the inverse Compton process. The inverse Compton process can be pictured as radiation by the electrons

Correction

In the News and Views article "Bugs are Beautiful" (**250**, 533; 1974), the abbreviation AISB stands for Artificial Intelligence and Stimulation of Behaviour Group; the published explanation is incorrect.

on account of their acceleration in the electric fields of the electromagnetic radiation through which they move, whereas synchrotron radiation is caused by the acceleration associated with spiralling round quasi-static magnetic field lines. Large magnetic fields, considerably in excess of 100 gauss, are needed to ensure that the synchrotron process is dominant, and such fields are not easy to come by with some models of the 'explosive event'. What Colvin has done is to suggest a two-stage process for generating the radiation from QSOs that is effective even with magnetic fields as small as 30 gauss.

The basic fact made use of in the theory is that for some radiation frequencies a plasma containing a magnetic field has a refractive index greater than 1. Highly relativistic particles therefore move through it at a speed exceeding the speed of light in the medium. In consequence they produce Čerenkov radiation—the electromagnetic equivalent of the shock wave generated in air by supersonic aircraft. Colvin estimates that under QSO conditions the Čerenkov photons could have a characteristic frequency of 10^8 Hz. The number of photons could be sufficient for them to make the relativistic electrons of lower energy then undergo inverse Compton energy loss and produce visible radiation of the intensity that is observed.

Strength functions in rare earths

from P. E. Hodgson

ACCORDING to the simple single-particle model of nuclei each nucleon occupies a state with definite energy and quantum numbers, and these are the eigenstates of a one-body potential. Such states are selectively populated by one-nucleon transfer reactions, and a study of these reactions enables the energies of the single-particle states to be determined.

Thus, for example, a (d,p) reaction simply puts a neutron into one of the unoccupied single-particle states of the residual nucleus, so that the energy spectrum of the emitted protons reflects the energies of the unoccupied states in the residual nucleus.

In practice such a simple result is only found even approximately for the low-lying states in reactions depositing a nucleon into a closed shell nucleus. In other cases the single-particle strength is distributed over a range of excitation energies. This takes place in two different ways; either the single-particle strength can be 'spread' over a large number of states in the residual nucleus according to a Lorentzian distribution or it can be 'fragmented' by

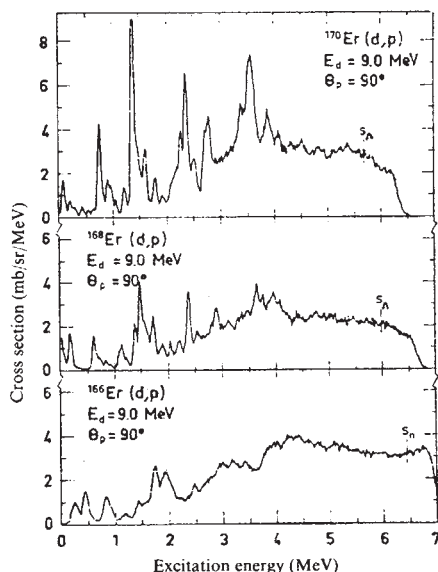


Fig. 1 Energy spectrum for the (d,p) reaction at 9 MeV on three isotopes of erbium.

residual interactions that are not taken into account by the one-body potential. The latter can result from a coupling of the single-particle states to vibrational degrees of freedom or from coupling between the one and three quasi-particle states. The spreading width is expected to increase with excitation energy while the fragmentation width remains almost constant.

It is important to try to find out the relative importance of spreading and fragmentation, for only if the fragmentation is less than the spreading is it possible to identify and assign quantum numbers to the higher states.

This problem has recently been studied in the rare earth region by Back, Bang, Bjørnholm, Hattula, Kleinhertz and Lien (*Nucl. Phys.*, **A222**, 377; 1974). They irradiated several isotopes of the rare earth nuclei gadolinium, dysprosium, erbium and ytterbium with 9 MeV deuterons and measured the energy distribution of the outgoing protons. Plotted on an appropriate energy scale this gives immediately a measure of the distribution of the neutron single-particle strength, or neutron strength function as it is called. A typical result, that for erbium, is shown in Fig. 1; the peaks in the spectra move towards lower energies for the heavier nuclei, which is what would be expected from the Nilsson model, and that there is structure below about 4 MeV but no structure at higher energies.

The theoretical cross sections for these reactions were calculated using the distorted wave theory of stripping reactions to single particle states in deformed nuclei. The wavefunctions were calculated as the eigenvalues of a deformed Saxon-Woods potential. To

enable the results of this calculation to be compared with the data the peaks were broadened by multiplying by a Lorentz factor. The results for erbium are shown in Fig. 2a and show qualitative agreement. The cumulative cross section (Fig. 2b) is in good accord with the data, indicating that the total neutron strength is preserved and that higher order processes are relatively unimportant. It is, however, difficult to make unique quantum number assignments to the peaks at higher energies.

This work gives a remarkably clear picture of the behaviour of neutron single-particle strengths as a function of excitation energy, and shows that both spreading and fragmentation take place.

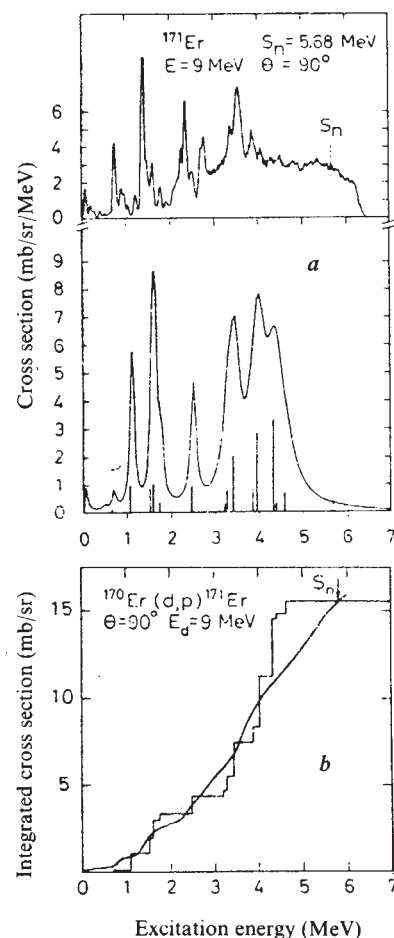


Fig. 2 a, Experimental (upper) and theoretical (lower) differential cross sections calculated in a deformed Saxon-Woods potential. The vertical lines represent the total stripping strength of the rotational band corresponding to the deformed single-particle state. The smooth curve represents this strength distributed according to the Lorentz factor. b, Comparison of the cumulative integrated experimental cross section and the theoretical cross section for energies below the neutron separation energy S_n .

articles

Biological significance of the intermolecular crosslinks of collagen

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Current theories of the crosslinks stabilising the collagen fibre are based on the isolation of compounds from borohydride-reduced collagen. What is the evidence for their existence in vivo and what, if any, is the physiological significance of these proposed crosslinks both in normal and pathological conditions?

THE recent advances in the biochemistry of collagen are due in part to its ubiquitous nature, as it exists in various forms specifically tailored to the particular function of each body tissue, and in part from the realisation that it is not completely metabolically inert but plays an important role in connective tissue disorders.

The special properties that enable collagen to act as the major supporting framework of the body are largely dependent on the high structural stability of the collagen fibres. Collagen fulfils this function by means of its unique molecular configuration, the highly specific alignment of the molecules during extracellular aggregation and finally by the formation of covalent crosslinks^{1,2}. The latter are particularly important in conferring on the fibres the high tensile strength and resistance to chemical attack necessary for their function. A malfunction, genetic or otherwise, in any one of the complex sequence of events leading to the formation of the final crosslinked fibre could result in an impairment of the fibre strength. There are a number of connective tissue diseases in which the clinical symptoms suggest a crosslinking defect. Furthermore, the extent of crosslinking may be important in affecting the catabolic rate in the more common conditions involving fibrosis and degeneration.

The initial stage in the formation of the intermolecular bonds is the oxidative deamination of specific lysine or hydroxylysine residues located in the nonhelical regions at both the N- and C-terminals of the molecules by a copper-dependent enzyme, lysyloxidase³. The aldehydes produced are then believed to condense with reactive groups, predominantly the ϵ -amino groups of hydroxylysine, situated in the helical region of neighbouring molecules thus forming a network of intermolecular bonds which were assumed to be of the aldimine type²⁻⁵. The lability of the aldimine bonds precluded the direct investigation of the compounds and the technique of mild reduction of the fibres with borohydride was therefore used to render the crosslinks stable to the hydrolytic procedures necessary for their isolation. All of the major reduced components present in borohydride-treated collagen have now been characterised and from the known action of borohydride, assumptions were made as to the form of these components that are present in the native fibre.

Early studies using radioactively-labelled lysine and lathyrig agents such as β -aminopropionitrile and α -amino β -thiols indicated that the reducible compounds were derived from lysine-aldehydes and were therefore implicated in the intermolecular crosslinking process⁶. Despite these indications, however, the identification of compounds, or even crosslinked peptides containing these compounds, from borohydride reduced collagen does not constitute proof that their nonreduced forms act as intermolecular bonds *in vivo* since the initial condensation reaction could have been promoted by the alkaline reducing conditions.

Our aim here, therefore, is to review critically the

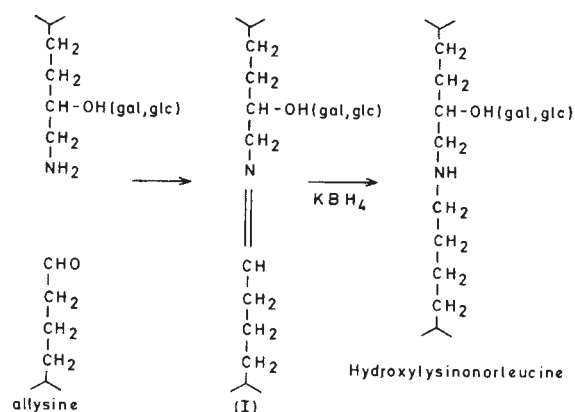


Fig. 1 Biosynthesis of dehydro-hydroxylysinonorleucine (I).

components isolated from borohydride-reduced collagen in relation to the physiological role of their nonreduced forms in the fibre. The influence of these compounds on the physical properties of the fibre will be considered particularly regarding the changes that occur with age, and in pathological conditions.

Components of reduced collagen

Hydroxylysinonorleucine. This compound was the first reduced component, believed to represent a reduced intermolecular crosslink, to be isolated and characterised⁷. Its presence in reduced fibres but not in solutions of monomeric collagens, and the lability of the native fibre to dilute acids, heat, and α -amino β -thiols, indicated that the nonreduced form existed as a labile intermolecular crosslink⁸. Subsequent characterisation after isolation from borohydride-

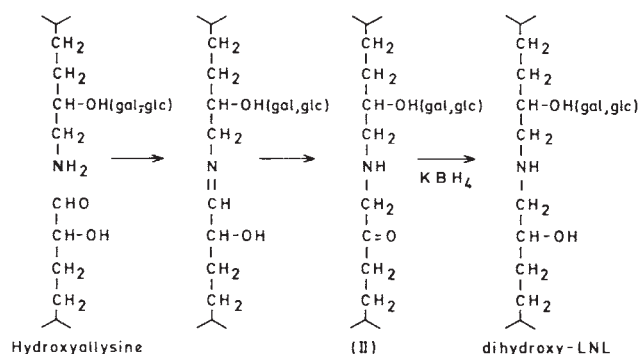


Fig. 2 Biosynthesis of hydroxylysino-5-keto-norleucine (II).

reduced native collagen was consistent with its proposed derivation from the aldimine bond form⁷ (Fig. 1). Unfortunately, the lability of the nonreduced form of the compound precludes the isolation of a crosslinked H-peptide from skin collagen to confirm its existence *in vivo*. However, when derived from hydroxyallysine and lysine the bond is stabilised by undergoing the Amadori rearrangement (see below). It is therefore possible to demonstrate its existence *in vivo* by isolation from bone or cartilage collagen (unpublished data).

Recently, both galactosyl and glucosyl-galactosyl derivatives of the crosslink have been characterised after their isolation from alkaline hydrolysates of borohydride-reduced collagens⁹. The relative proportions of the mono- and disaccharide crosslinks varies with different tissues as does the total extent of glycosylation. The attachment of hexose does not seem to have a significant effect on the stability of the aldimine bond, but may facilitate aldimine bond formation by lowering the pK of the ϵ -NH₂ group.

Hydroxylysino-5-keto-norleucine. This compound designated syndesinol was reported to be a major component of borohydride-reduced tendon, bone and cartilage but was misidentified as an aldol derived from hydroxyallysine¹⁰. Later studies by mass spectrometry indicated that it was a reduced aldimine derived from hydroxylysine^{11,12} and this structure was confirmed by chemical synthesis¹². But the assumption that this compound exists *in vivo* as an aldimine was inconsistent with its unusual stability to heat, dilute acids and D-penicillamine^{6,13}. It was therefore proposed that migration of the double bond could take place to form the stable keto form by undergoing a spontaneous Amadori rearrangement¹⁴. This proposal was confirmed by a Smith degradation of the reduced compound¹⁵ isolated from bone: the tritium activity was found to be associated almost exclusively with the proline and hydroxynorvaline produced, thus indicating that the rearrangement must have been complete. Further studies have shown that the rearrangement is also complete in tendon and embryonic skin. The crosslink must therefore exist *in vivo* as hydroxylysino-5-keto norleucine (Fig. 2).

In common with the monohydroxy crosslink, glycosylated derivatives of di-hydroxylysino-5-keto-norleucine are present in varying proportions with different tissues^{9,16}. Since the crosslink exists completely in the keto form in the native fibre, clearly the carbohydrate moiety can only be attached to the remaining hydroxyl group of the hydroxylysine residue from the helical body of the molecule.

The stability of this crosslink suggested that it might be possible to isolate a crosslinked peptide from nonreduced cartilage collagen as described by Miller¹⁷ for the isolation of the corresponding peptide from borohydride reduced fibres. The peptide isolated gave an amino acid analysis consistent with a crosslinked dipeptide of α 1(II) CB4 and CB9, and on reduction revealed the presence of dihydroxylysino-5-keto norleucine¹⁸. Periodate cleavage and subsequent reduction of the reduced H-peptide produced two peptides with

analyses consistent with CB4 and CB9. Alkaline hydrolysis of the peptide indicated that about 20% of the crosslink was present as the glucosyl-galactosyl derivative.

The isolation of a crosslinked peptide from nonreduced collagen provides further evidence that this hydroxy-allysine derived component does exist *in vivo* acting as an intermolecular crosslink between two adjacent collagen molecules. From the order of the CNBr peptides of cartilage collagen α 1(II) chains determined by Miller¹⁹ it is obvious that the crosslink occurs between the N-terminal telopeptide and a hydroxylysine residue in the helical region of the molecule such that the two molecules are overlapped as predicted by Hodge and Petruska²⁰.

It is conceivable that the acid conditions of the CNBr cleavage catalysed the Amadori rearrangements of the aldimine bond and hence, that the isolation of a peptide containing the crosslink in the keto form is not proof of its existence in the native fibre. But the previous experiment in which the native fibre was reduced under alkaline conditions confirmed that the crosslink exists entirely in the keto form. Thus, taken together these two experiments provide direct proof that hydroxylysino-5-keto-norleucine acts as an intermolecular crosslink in the collagen fibre under physiological conditions.

Histidino-hydroxymerodesmosine. This component, present in high proportions in borohydride-reduced skin and tendon⁶, has recently been shown to have a structure capable of linking three or four peptide chains²¹. The component isolated is presumed to be formed by the Michael addition of histidine to the α,β -unsaturated double bond of the intramolecular aldol, and subsequent condensation of the aldehyde moiety of the aldol with hydroxylysine; the aldimine formed is stabilised by borohydride reduction thus permitting isolation of the compound (Fig. 3).

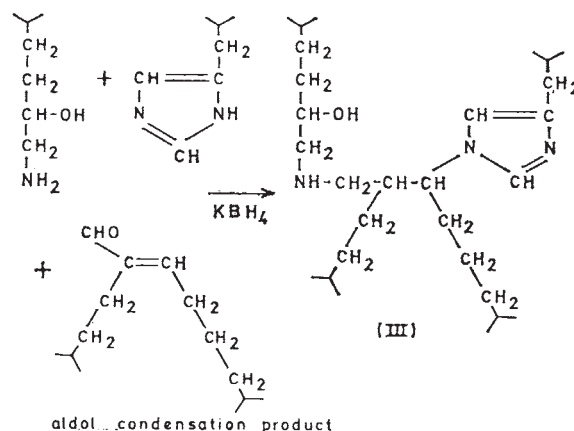


Fig. 3 Formation of histidino-hydroxymerodesmosine (III).

Based on the structure of this compound, histidino-hydroxymerodesmosine, it was proposed that the compound existed before reduction as the aldimine bond form²¹. Direct confirmation by the isolation of a crosslinked peptide is not possible owing to the extreme lability of the nonreduced form. Indeed, it was these earlier studies on the stability of the presumed crosslink in native fibres that called into question the existence of this complex component under physiological conditions^{15,22}. In particular, fibres pretreated with acid phosphate not only failed to form histidino-hydroxymerodesmosine on borohydride reduction but also resulted in a concomitant increase in the amount of the reduced intramolecular aldol. The proportion of the dehydro-hydroxylysino-5-keto norleucine was unaffected under these conditions. These results imply that cleavage has occurred of both the aldimine bond and the Michael addition product.

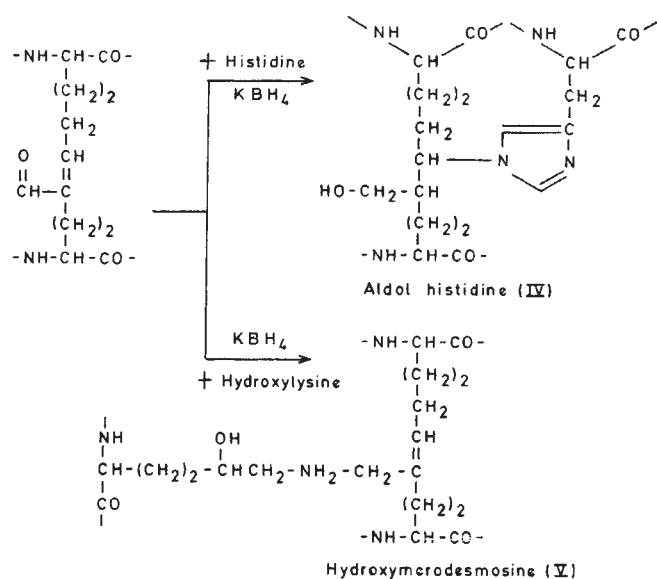


Fig. 4 Formation of aldo-histidine (IV) and hydroxymero-desmosine (V).

But, unlike the aldimine bond linking the hydroxylysine residue, the Michael adduct forms a covalent bond which is not stabilised on reduction with borohydride but is a stable entity *per se*, as evidenced by the resistance of the histidine-aldol bond to cleavage by acid or base hydrolysis during its isolation.

The most likely explanation is that the Michael addition does not occur under physiological conditions but is a base-catalysed reaction brought about during reduction with borohydride²². Any disruption of the precise three-dimensional arrangement of the molecules in the fibre (such as freeze-drying) was found to partially inhibit the formation of this compound on borohydride reduction. Thus, although it is conceivable that some noncovalent stabilisation (for example, hydrogen bonding as occurs in the active site of some enzymes^{23,24}) may be derived from the adjacent functional groups in the native fibre, the results suggest that the proposed nonreduced aldimine form of histidino-hydroxymero-desmosine does not exist as an intermolecular crosslink *in vivo*.

Minor reducible components

Lysinonorleucine. The small amounts of this compound in reduced native collagen^{25,26} probably reflect incomplete hydroxylation of the lysine residues in the helical portion of the molecule that participate in aldimine bond formation. Indeed, when hydroxylation of the lysines is partially inhibited, as in vitamin C deficiency, then a higher proportion of this component is present²⁷. It is unlikely that the presence of such small amounts of this crosslink makes a significant contribution to the stability of the collagen fibre.

Aldo-histidine and hydroxymero-desmosine. These two reduced components have been isolated in small amounts and have been proposed as intermediates in the formation of histidino-hydroxymero-desmosine^{28,29} (Fig. 4). Since the Michael addition of histidine to the aldol is believed to be promoted by borohydride it is probable that aldo-histidine is also an artefact. In view of the apparent instability of the nonreduced form of hydroxymero-desmosine and its possible conversion to the histidine derivative on reduction the role of this crosslink is difficult to assess, but the effect of the small proportion detectable is likely to be minimal.

Hexosyl-lysine and hexosyl-hydroxylysine. The characterisation of these reducible components^{30,31} which are present only as minor constituents in young tissue but which apparently increase with age demonstrated that they are derived from the condensations of lysine and hydroxylysine with glucose and mannose (Fig. 5). Their structure clearly indicates that these compounds can neither be involved in collagen crosslinking nor can they bind collagen to glycoprotein since in the latter no reducing group is available for the condensation reaction. Although the presence of these compounds might indicate a progressive increase with age in the binding of polysaccharides to collagen, the physiological implications, if any, of these observations are as yet unknown.

Tissue differences

From the foregoing discussion it is apparent that of the components isolated from reduced collagen of young tissues, only hydroxylysinonorleucine and dihydroxylysinonorleucine are derived from intermolecular crosslinks present *in vivo* in significant amounts. Clearly, the type of crosslink formed is governed by the extent of hydroxylation of the lysine residues in the N- and C-terminal nonhelical regions. In skin these residues are not hydroxylated³²⁻³⁴ leading to the formation of dehydro-hydroxylysinonorleucine whereas in bone and cartilage hydroxylation is virtually complete^{35,36} resulting in the formation of hydroxylysino-5-keto-norleucine. In tendon collagen, the lysine is about 50% hydroxylated giving rise to both these crosslinks in roughly equivalent amounts.

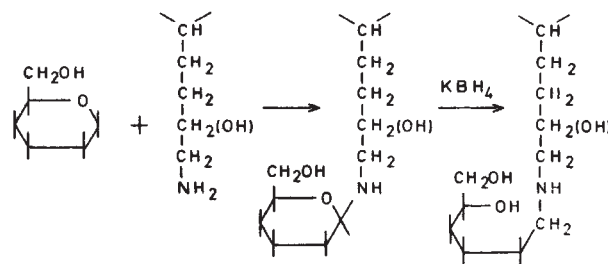


Fig. 5 Formation of hexosyl-lysines and -hydroxylysines.

The influence of these different crosslinks on the properties of the tissue should be reviewed in the context of their physiological function. In the absence of the lysine-derived aldehydes in both experimentally induced lathyrism and copper deficiency, the collagen fibres have very little tensile strength and are completely soluble in neutral salt buffers². The formation of the intermolecular covalent crosslinks provides a high tensile strength to the fibre but this does not necessarily render the collagen insoluble. Thus the aldimine component, dehydro-hydroxylysinonorleucine, provides a high tensile strength to rat tail tendons but the fibres are completely soluble in dilute acetic acid owing to the chemical lability of this bond. For skin collagen this crosslink can account, at least in part, for the tensile strength but not for the high proportion of insoluble collagen. By contrast, in the highly insoluble collagens such as bone and cartilage, the major reducible intermolecular bond *in vivo* is the stable crosslink, hydroxylysino-5-keto-norleucine, and its presence could therefore account for the insolubility of these tissues. Similarly embryonic skin³⁷ and granulation tissue³⁸ possess a high proportion of this crosslink and contain very insoluble collagens. But the possibility cannot be ruled out that other factors such as the presence of glycosaminoglycans may contribute to the solubility of these types of collagen

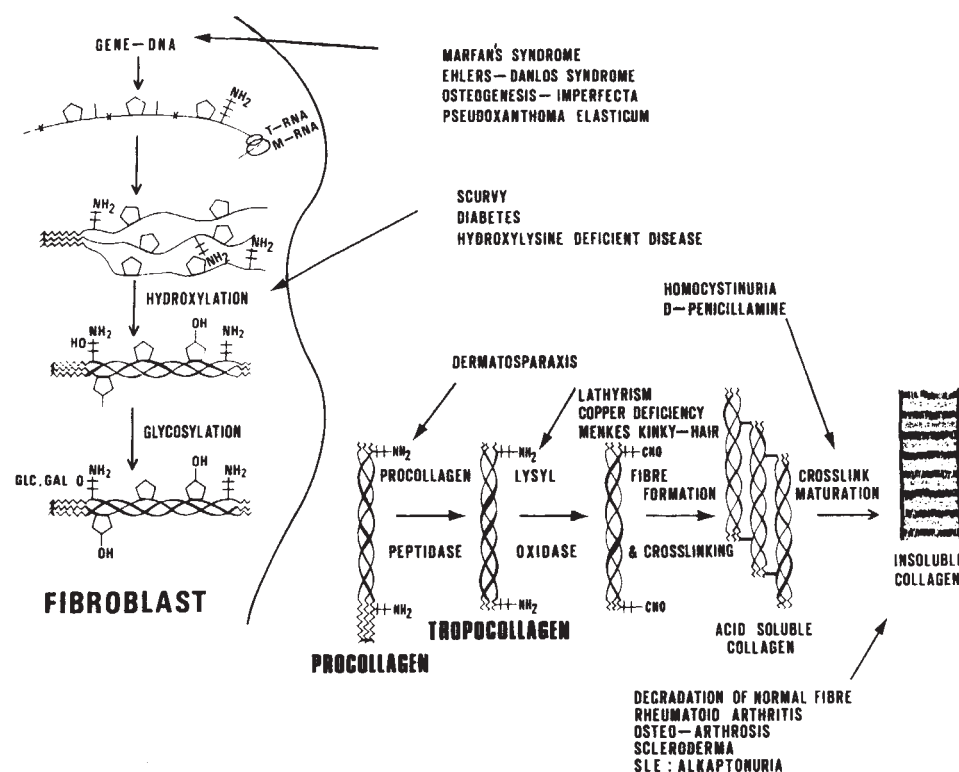


Fig. 6 Schematic representation of the series of reactions in the biosynthesis of the interstitial collagen fibres depicting the possible stages at which defects could occur resulting in genetic disorders. The dominantly inherited disorders, Marfan's syndrome, Ehlers-Danlos Syndrome, osteogenesis imperfecta and pseudoxanthoma elasticum suggest a primary structure defect⁴³. Scurvy results from a vitamin C deficiency leading to inefficient hydroxylation⁴⁷. Diabetes has been reported to result in excess hydroxylation⁴⁸. Hydroxylysine deficient disease is due to the absence of lysyl hydroxylase⁴⁵. Dermatosparaxis results from the absence of procollagen peptidase^{47,48}, lathyrism is due to inhibition of lysyl oxidase²¹ and Menkes kinky hair syndrome to copper deficiency⁵⁰, an essential co-factor of lysyl oxidase². Homocystine⁵⁰ and D-penicillamine act by cleavage of the labile aldimine crosslink.

and any proposed relationship between the nature of the crosslink and solubility must, in the absence of further experimental evidence, remain unconfirmed. As discussed below, the reducible crosslinks are known to decrease with age and it is important to emphasise that these observations only apply to young growing tissue.

Age-related changes

It is well known that with increasing age collagenous tissues become increasingly more stable to external influences³⁹. For soft tissues such as skin, there is a progressive decrease in the amount of collagen extractable by acetic acid, a fact that is incompatible with the presence solely of the labile aldimine type of intermolecular bonds. Studies of the reducible components present in tissues covering a wide range of ages showed that no new compounds other than the hexosyl-lysine derivatives are formed during the ageing process. The variations with age in the proportions of the reducible crosslinks were found to parallel the rate of growth¹⁴. Thus, there is an initial rapid rise in their amounts during the laying down of new collagen but this is followed by a gradual decrease so that at maturity the reducible crosslinks are virtually absent. It is during this latter period that the collagen fibres become increasingly insoluble whilst retaining their tensile strength. To account for these facts, it was proposed that the reducible crosslinks act only as intermediates and are converted during the maturation process into a nonreducible and, certainly in the case of the aldimine type compound, a more stable form.

One possible modification of the crosslinks that could account for the observed results is by reduction *in vivo* to form compounds identical to those produced by the borohydride treatment. Such a reaction is known to occur in elastin, resulting in the formation of lysinonorleucine from its aldimine form⁴⁰ but a similar analysis of old nonreduced

collagen failed to reveal any *in vivo* reduced forms²³. Later, Mechanic *et al.*⁴¹ proposed from mass spectral data that some 25 to 50% of the intermediate crosslinks in bone and dentine collagen are reduced *in vivo* and supporting evidence was derived from the *in vitro* ageing studies of Deshmukh and Nimni⁴². A more recent study⁴⁴ using several different techniques has, however, confirmed the earlier report that reduction *in vivo* of the intermolecular bonds does not occur in collagen. Thus the nature of the stable crosslinks present in the insoluble mature tissue remain to be elucidated, although based on the evidence now available the most likely explanation is that these stable bonds are derived in some way from the reducible crosslinks. Hence, it is not necessary to propose a continuous increase in the number of crosslinks, with all the attendant difficulties of enzyme reactions within the fibre: rather a spontaneous conversion of the reducible intermediates to the stable mature form would account for the observed facts.

Collagen diseases

A number of heritable connective tissue disorders suggest, at least from the clinical symptoms, that the disorder might be a defect in the crosslinking mechanism (Fig. 6). The Ehlers-Danlos^{43,44} syndrome in which the skin is hyperextensible, the Marfan's⁴³ syndrome in which the aorta frequently ruptures, and osteogenesis imperfecta⁴³ in which the bones are extremely brittle, all strongly suggest that the disorders could be defined in terms of the molecular biology of collagen.

Such definitions have recently been achieved with some recessively inherited disorders. In a type of Ehlers-Danlos syndrome (Type VI) the defect was shown to be an absence of lysyl hydroxylase resulting in a collagen deficient in hydroxylysine⁴⁵. Since hydroxylysine is an essential component of all the collagen crosslinks so far identified it is

not surprising that there was a consequent lack of cross-linking⁴⁶ consistent with the clinically observed tissue fragility. Another enzyme deficiency occurs in Ehlers-Danlos type VII in which the low level of the procollagen peptidase activity results in the presence of procollagen during fibre formation⁴⁷. The defect may be a milder form of dermatosparaxis described in cattle in which the presence of the extra nonhelical regions disorganises fibre formation resulting in inefficient crosslinking and hence a fragile fibre^{48,49}. The disruption and fragmentation of the large arteries in Menkes kinky hair syndrome is probably due to the low intestinal absorption of copper⁵⁰ the absence of which is known to lead directly to inhibition of cross-linking in both collagen and elastin⁵¹.

Although some success has been achieved with the recessively inherited disorders, the basic defect in the dominantly inherited disorders, Ehlers-Danlos, Marfans and osteogenesis imperfecta, is less forthcoming. Solubility and tissue culture studies suggest a slightly decreased level of crosslinking^{52,53} but there is no dramatic change in the lysyl oxidase activity⁵⁴ or the type of crosslink in these disorders⁵⁵.

In the more common debilitating connective tissue diseases a rapid proliferation of collagen often occurs resulting in the malfunction of certain tissues as with skin in scleroderma, the joint capsule in osteoarthritis, the synovial membrane in rheumatoid arthritis, hypertrophic scar, cirrhosis and arteriosclerosis. The increased accumulation of collagen may be due either to increased synthesis, or to a decrease in catabolism, the latter being possibly due to inhibition of the collagenases or to the type of crosslink being synthesised. In scleroderma the newly synthesised collagen possess the labile crosslinks of young skin⁵⁶ and in rheumatoid synovium and granulomas⁵⁸ the stable keto crosslink is formed, results which may influence rate of catabolism and subsequent treatment of the disease.

In conclusion it may be stated that at least some of the reduced components in borohydride reduced collagen exist in their nonreduced form as intermolecular crosslinks determining the physical characteristic of the fibre during the rapid growth and remodelling process. Further, some correlation between the type of crosslink and the pathology of the tissue is beginning to emerge. The next stage should be directed towards the elucidation of the nature of the stable nonreducible intermolecular crosslinks present in mature collagen in order to further elucidate the nature of collagen in health and disease.

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Infrared stars or infrareddened stars?

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Extreme interstellar reddening, accompanying visual absorption of order 100 magnitudes, is still a viable explanation for infrared objects like Becklin's star in spite of recent claims to the contrary. It is unnecessary at this stage to invoke a new phase of protostellar evolution to account for them.

SOURCES in regions of star formation which do not correspond to any optical stars are among the most interesting new types of object found by infrared astronomers. The best known of these¹ is variously known as BN, Becklin's object and Becklin's star. It has a colour temperature near 600 K. Infrared sources which may be similar objects have subsequently been found in other star-formation regions, notably NGC 2264 (ref. 2), CasOB6(W3) (ref. 3) and the R CrA association⁴. The source in W3—called IRS 5—has a colour temperature as low as 200 K, but the other two are less extreme than Becklin's star and lie near the apexes of small conical nebulae.

Becklin and Neugebauer proposed¹ that their source was a young object—perhaps a protostar—buried in a dust cocoon and that its infrared emission derived from re-radiation by the dust cloud. This explanation was widely accepted until 1971 when Hyland and ourselves⁵ proposed that the infrared source was the photosphere of a very luminous star reddened by the presence of extreme quantities of dust. For a typical reddening law the visual absorption, A_V , must be about 70 mag.

At the time there were several pieces of evidence to support that view. The colours in the 1.6 to 3.5 μ m region were those of a reddened hot black body rather than a cool black body and microwave observations of the Orion Nebula showed it to be sufficiently dense that A_V across it must be of order 200 mag for a normal dust to gas ratio. Further, Becklin's star is immersed in an extended 20 μ m source (KL, the Kleinmann–Low nebula) which is naturally accounted for as the thermal radiation of the absorbing dust. This nebula is optically thick at 20 μ m, also suggesting high visual extinction. Because the 2 μ m spectrum is featureless⁶, Becklin's star must have a spectral type earlier than G and later than about B; Penston *et al.*⁵ favoured an F star because stars of that spectral type having the required luminosity ($10^6 L_\odot$) are known in other galaxies.

In ref. 5 it was predicted that the ubiquitous silicates, found in emission in a separate extended source near the Trapezium cluster⁶, would produce an absorption feature in the 10 μ m spectrum of Becklin's star. The silicate absorption was indeed found by Gillett and Forrest⁷ who also reported an absorption band at 3.2 μ m due to water ice. Both these features are about 1 mag deep. Further support for the heavy reddening hypothesis was provided by infrared polarimetry. At most infrared wavelengths, and in particular within the silicate absorption band, Becklin's star is the most strongly polarised source known. A typical value for the linear polarisation is 10% (refs 8–10); scattering by dust particles is the mechanism usually invoked to explain such large polarisations.

In spite of these accumulated supporting data, the heavy reddening hypothesis has been criticised recently, and even held to be no longer tenable. Becklin, Neugebauer and Wynn-Williams found Becklin's star at 20 μ m to be brighter by 1.5 mag than a hot reddened star fitted to the short wavelength data¹¹. They argued that dust accounted for the flux at 20 μ m and therefore may contribute strongly at all other wavelengths. They suggested that A_V is about 10 mag by comparison with the

galactic centre source which they took to have an A_V of 25 mag on the basis of its 2 μ m colours, and to have a silicate absorption twice as deep as that in Becklin's star.

Criticism has also come from Rieke, Low and Kleinmann¹² who made high resolution maps of the region at various wavelengths in order to disentangle the contributions from BN and KL. By combining their derived fluxes with data at longer wavelengths, Rieke *et al.* deduced the luminosity of the KL nebula to be about $5 \times 10^4 L_\odot$. If the Kleinmann–Low nebula absorbs most of the energy of the underlying star it must essentially have the luminosity of that star. The figure $5 \times 10^4 L_\odot$ is too low to be reconciled with the best available photometry of Becklin's star.

This situation is worsened by the discovery¹² that Becklin's star is only one of several similar sources that cluster within the KL nebula. Some of these sources were found by Wynn-Williams and Becklin¹³ and given different designations. In Table 1 we list all the infrared sources known in the Orion Nebula. There are two distinct types: most are compact objects less than 2 arc s diameter, but three are extended and can be attributed to thermal radiation from hot gas (free-free emission) or cool dust. It is not clear from present data whether two of the 'compact' sources, IRc4 and IRc5, really are compact or are small condensations in the Kleinmann–Low nebula. According to Rieke *et al.*, IRc2, 3, 4 and 5 are redder than Becklin's star and probably have deeper silicate absorptions.

It is important to clarify the essential issue underlying this controversy about the value of the absorption (which we will continue to measure by A_V while noting that it is in fact determined at 2 μ m). If A_V lies in the range 65 to 70 mag, the unreddened source can be explained by a normal star in the temperature range 5,000 to 12,000 K. Such stars are consistent with the 2 μ m spectrum. On the other hand if A_V is less than 45 mag the unreddened source has a colour temperature below 1,500 K and the radiation must originate principally from dust grains. No consistent model exists with A_V between 45 and 65 mag. In the second case Becklin's star may be an example of a new class of prestellar object. On the evidence available so far, must such new objects be invoked or can an explanation in terms of normal stars be retained?

It seems most unlikely that A_V can be as low as the 10 mag suggested by Becklin *et al.*¹¹, since there is no sign of anything like such strong silicate or ice absorption in stars, such as Cyg OB2 12 (ref. 14) and MWC349 (ref. 15), with $A_V \sim 10$. The comparison with the galactic centre is of dubious weight since the 2 μ m galactic centre source (on which A_V is based) and

Table 1 Infrared sources in the Orion Nebula

Type of source	Designation ref. 12	Probable ref. 13 A_V (mag)	Notes
Definitely compact (stars)	IRc1	BN	70
	IRc2		100
	IRc3	IRS 3	160
		IRS 2	40
		IRS 4	? near NA
Compact or dust concentrations?	IRc4	core of KL	≤ 200
	IRc5		? seen only at 21 μ m
	IRe2	KL	50–200
Extended	IRe1		small
		NA	small free-free component Trapezium

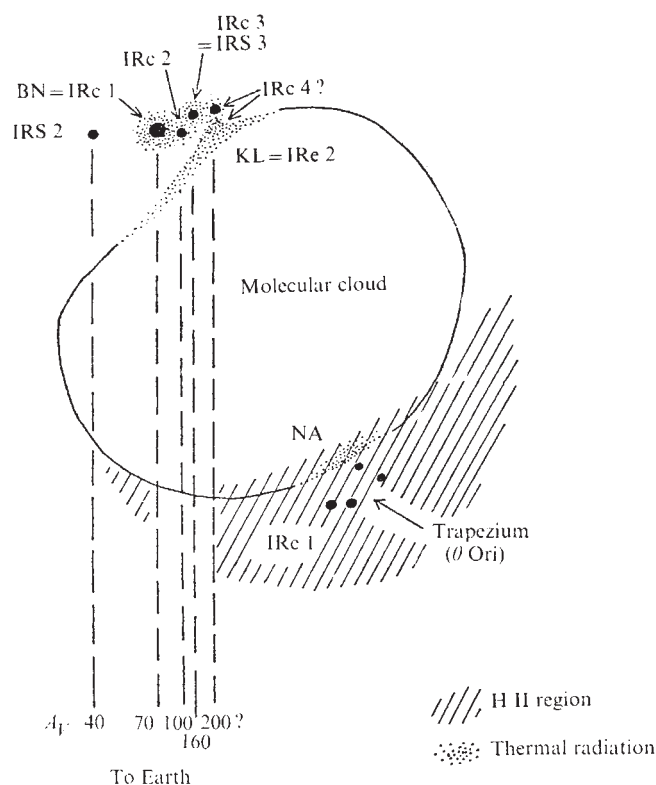


Fig. 1 Schematic diagram of M42 sliced through position angle 145° – 325° . The solid boundary to the neutral cloud is intended to represent not the limit of the material but a contour above which the density is very high. IRc5 and IRS 4 are not plotted because of the uncertainty of their nature.

the $10\ \mu\text{m}$ source (with silicate absorption) have different structure.¹⁶ In general an extended source will tend to show smaller absorption at shorter wavelengths if the absorbing medium is clumpy. Further, although the ratio of the equivalent widths of the silicate bands in the galactic centre and Becklin's star is about two, a more sophisticated analysis¹⁷ including the effect of emission shows the column density of silicates to be more nearly the same.

The choice between an A_V of 30 to 45, consistent with Rieke *et al.*'s model of a cluster of protostars completely enveloped in the Kleinmann–Low nebula, and an A_V of about 70 is not so easily made. We wish to point out that the latter is by no means untenable and that only slight modifications to the original version of ref. 5 are necessitated by the large number of new data. We must now explain (i) the $20\ \mu\text{m}$ excess and (ii) the discrepancy in luminosity between the KL nebula and the cluster of stars.

The $20\ \mu\text{m}$ excess

There is no difficulty in finding a mechanism to produce the $20\ \mu\text{m}$ excess. Re-radiation by cool ($\sim 200\ \text{K}$) dust, or possibly free-free emission by circumstellar gas, can provide the necessary $20\ \mu\text{m}$ flux without significantly affecting the near infrared colours. An explanation based on dust at such a temperature would not require the source to be more extended than 1–2 arc s. Dust re-radiation would also tally with the silicate emission implied in ref. 17.

On the other hand the dereddened 5– $20\ \mu\text{m}$ colour and the gradient of the 8– $13\ \mu\text{m}$ continuum fit a free-free slope. Such a free-free component could not, however, originate in an ionised hydrogen shell without the simultaneous emission of a Brackett γ line, sufficiently intense to have been detected by Penston *et al.*⁵. Cool ionic free-free, as considered by Dyck and Milkey¹⁸, is an outside possibility.

The luminosity

Because insufficient luminosity is re-radiated by the KL nebula, it cannot completely surround the stars. We therefore now envisage a cluster lying at the far side of the Orion Nebula just as the Trapezium cluster lies on the near edge (Fig. 1). There would not necessarily be any manifestations at optical wavelengths of the presence of such luminous stars outside the nebula and essentially unobscured from some aspects. In particular any Lyman continuum radiation is soaked up by the circumstellar gas so these stars would be incapable of ionising more distant nebulosity.

It is relevant that very luminous F and G type stars are seen associated with star-formation regions in the Large Magellanic Cloud¹⁹. Luminosities run up to $10^6 L_\odot$ as in HD268757 which is associated with the nebula N83. Thus a star of $10^5 L_\odot$ is by no means exceptional. We are not privileged to examine the star-formation regions in our own galaxy face on; these extremely bright stars might well be hidden behind dust clouds.

A more severe problem arises in the case of IRS 5 in W3 if this source too is to be explained as a highly reddened star. To account for the depth of the silicate feature¹⁷ we need only assume the ratio of silicate to total absorption to differ marginally from that of Becklin's star. But the near infrared photometry implies a total luminosity of $\sim 3 \times 10^6 L_\odot$ even if we choose what seems to be the more reliable distance of 2 kpc for Cas OB6 (ref. 20) rather than the value of 3 kpc used to date in discussions of this infrared star. Like Becklin's object, the reddened star must have a $20\ \mu\text{m}$ excess. A multiple system of very luminous stars could account for this object also; an alternative is a truly remarkable (and possibly unstable) single object of the type discussed by Hoyle, Solomon and Woolf²¹. That W3 IRS 5 must also lie at the back of its absorbing cloud is implied by the $100\ \mu\text{m}$ flux reported for this source²² which at 2 kpc corresponds to a luminosity of $5 \times 10^6 L_\odot$. Most or all of the flux can be accounted for by other sources in W3 (such as IRS 2 of ref. 3), so IRS 5 may lie so far back of the absorbing cloud that there is essentially no re-radiation.

The Kleinmann–Low nebula

Returning to discuss the details of the Orion Nebula sources, we note that in our model the Kleinmann–Low nebula is also seen through dust and could have an A_V as high as 200 mag. Gillett and Forrest⁶ suggested that the optical thickness of the silicate absorption in the KL nebula is much greater than in Becklin's star, implying a higher A_V . The polarisation is also higher¹⁰. Unfortunately, the interpretation of these data is complicated. The two source picture (BN+KL) was extant when these

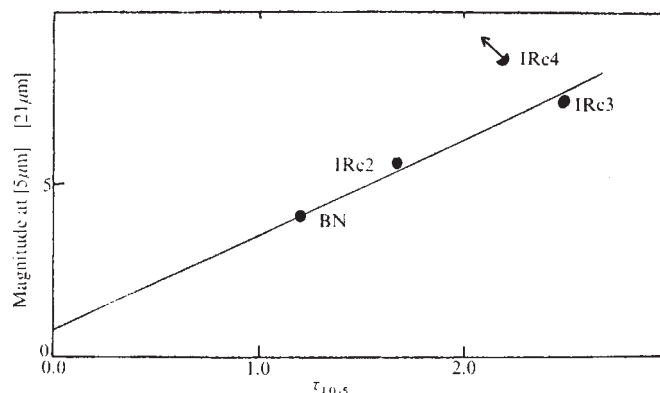


Fig. 2 A plot of the $5\ \mu\text{m}$ to $21\ \mu\text{m}$ colour index of the compact infrared sources in M42 against the depth of the silicate feature below a reddened continuum fitted at 5 and $21\ \mu\text{m}$. The data are from ref. 12. This correlation can be explained if the sources have similar intrinsic 5– $21\ \mu\text{m}$ colour indices and are reddened in proportion to the silicate absorption.

observations were made, but now we know of many sources. Establishing how much each source contributes to each observation is impossible without a very accurate knowledge of the size, profile, separation and position of the beams at the time of observation. The uncertainty has only minor effect on the results for Becklin's star itself, but could greatly distort the results for the extended source.

Observational tests

It is not easy to distinguish between the 10,000 K, 70 mag model and the $\sim 1,200$ K, ~ 30 mag model. One method would be to measure interferometrically the diameter of Becklin's star at $5\ \mu\text{m}$ where the long wavelength component is negligible and scattering should not much increase the apparent size. A baseline of 500 m would be needed.

An alternative would be to deduce the relationship between A_V and the depth of the silicate or ice absorption. For example more stars like Cyg OB2 12 and MWC349, and possibly the galactic centre, should be observed in this way. The form of the interstellar absorption curve in the near infrared should follow from these observations as well as the depths of selective absorption features. If infrared sources like those in NGC2264 and R CrA do fit into this scheme they may be most valuable as intermediate cases since the optical nebulae probably caused by reflection^{4,23} indicate the presence of hidden stars. The ratios of continuous to selective and of optical to infrared absorption are unlikely to be constant throughout the Galaxy, so this process is best done for the immediate vicinity of the Orion Nebula, where there may be no suitable sources. From the data given in ref. 12 we may, however, deduce a relationship between the reddening at $5\ \mu\text{m}$ and the apparent depth of the silicate feature. In Fig. 2 we have plotted the $5\ \mu\text{m}$ to $21\ \mu\text{m}$ colour index in magnitudes against the optical depth at $10.5\ \mu\text{m}$ in the silicate band. The tight correlation exhibited by BN, IRC2 and IRC3 is further evidence for the presence of reddening. The limit on IRC4 does not fit this correlation, but we have already noted (Table 1) that this source may be of a different nature. It is interesting that IRC3 seems to possess double the absorption found in BN. The intersection of the best fit line with the $[5\ \mu\text{m}]$ – $[21\ \mu\text{m}]$ axis gives the intrinsic colour of the sources, assuming them all to be similar; this is seen to be small. For a typical theoretical reddening law $A_V \sim 50E_{(5)-(21)}$, but this seems to be an overestimate since it suggests $A_V > 100$ mag for BN, and this figure could be further raised if there is any silicate absorption at $21\ \mu\text{m}$. Improved data might lead to a more reliable diagram of this kind. Essentially identical conclusions follow from interpreting Fig. 1 of ref. 24 in the same way.

A final test would be to obtain a better $2\ \mu\text{m}$ spectrum of

Becklin's star to search for normal stellar features, for example Brackett γ . With the improvement in signal/noise ratio likely to be brought about by the introduction of InSb detectors, this is an attractive proposition.

Even if Rieke *et al.*'s model for the Orion sources is correct, absorptions with $A_V \geq 30$ mag shroud any optical counterparts from our view. Such dense clouds and high absorptions were unanticipated before the advent of infrared astronomy: they are now a recognised part of the process of star formation.

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Catalytic head assembling protein in virus morphogenesis

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About 250 molecules of a 'scaffolding protein', not found in mature phage, catalyse the assembly of about 420 coat protein subunits into a precursor shell containing both proteins: in concert with DNA encapsulation, all the scaffolding molecules exit from the precursor shell to take part in further rounds of the same process.

SINCE Fraenkel-Conrat and Williams demonstrated that tobacco mosaic virus could be reconstituted from its purified protein and nucleic acid components¹, it has been widely believed that virus parts specify their own correct assembly. Caspar and Klug² provided a geometrical model for the self assembly of spherical virus capsids as the aggregation of similar subunits into quasi-equivalent positions on the surface of an icosahedral shell. There is

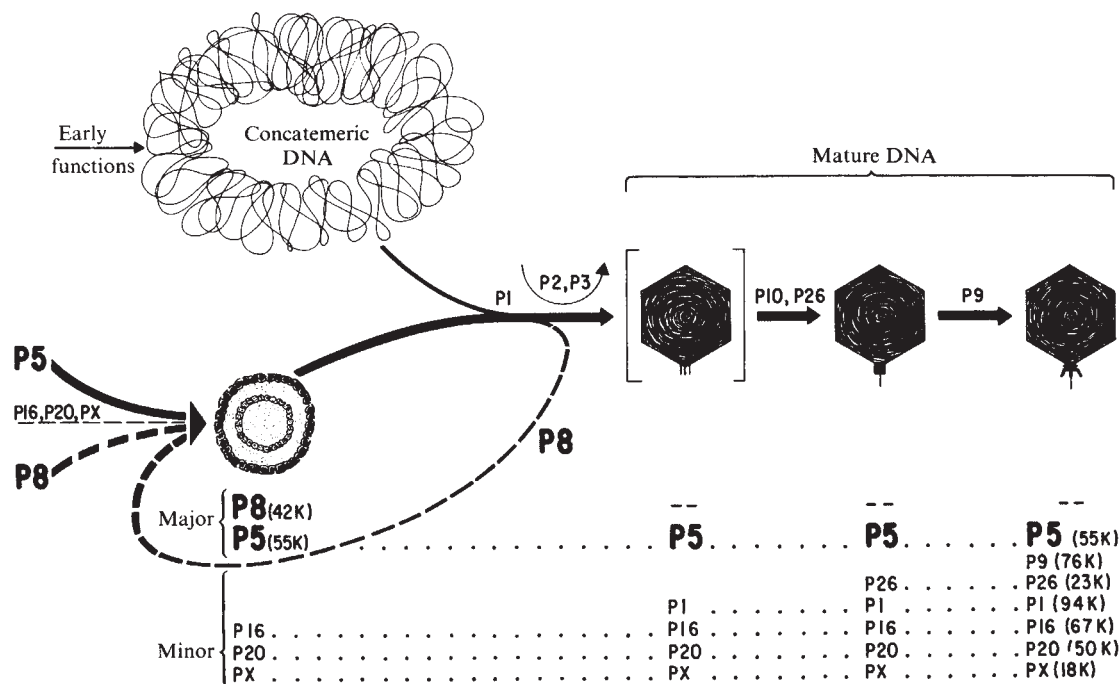


Fig. 1 Pathway of phage P22 head assembly and DNA encapsulation. This diagram summarises results from Botstein *et al.*¹⁷, King *et al.*¹⁸ and this article. A prohead shell lacking DNA is the first identifiable structure in the pathway. It contains two major protein species, the products of genes 5 and 8. (The products of genes 16 and 20, though ordinarily incorporated into proheads, are not needed for morphogenesis.) With the incorporation of the product of gene 1, and the function of the gene 2 and 3 products, the prohead encapsulates and cuts a headful of DNA from the concatemeric DNA molecule, and concurrently P8 exits from the particle without proteolytic cleavage. The newly filled head is unstable (it loses the DNA) until acted upon by the gene 10 and 26 products, after which the complete head is converted to a phage particle by addition of the tail parts (gene 9 protein) as shown by Israel *et al.*³⁷. P before a gene number refers to the polypeptide of that gene. The polypeptide chains found in each structure are listed below it, with their molecular weight shown in the last column. Brackets around the first filled head structure designate that it is unstable and loses DNA on isolation. The nature of the replicating DNA in P22-infected cells has been described by Botstein and Levine²²; Tye *et al.*¹⁶ have shown conclusively that P22 chromosome maturation proceeds by a "sequential headful cutting" mechanism³¹.

now strong evidence that at least simple viruses conform to this pattern, and can self assemble from the separated components of the mature virus³⁻⁵. Recently, however, experiments with the more complex bacteriophages λ and T4 have shown that some proteins are required for assembly which are absent from the mature virus⁶⁻⁹. In addition proteolytic cleavage^{5,8,10-12}, and even covalent fusion¹³ of structural proteins have been found to accompany the assembly of various viruses and bacteriophages. We describe here a protein that specifies the correct dimensions of a virus, catalytically, by temporarily complexing with the major coat protein to form a transient precursor shell of the virus. The dissociation of the protein from the precursor shell is associated with DNA condensation.

P22 is a temperate phage which infects the bacterium *Salmonella typhimurium*¹⁴. Its head is isometric, 600 Å in diameter, with a small spiked tail plate attached (Figs 1 and 2b), and contains double-stranded DNA of molecular weight 27×10^6 (ref. 15). The sequence present at one end of the chromosome is repeated at the other end to the extent of about 2%, and the genome is circularly permuted with respect to the chromosome so that the ends are not unique^{15,16}. The DNA metabolism of infected cells, the genetic control of virus assembly, and the protein composition of virus particles have been described in detail by Botstein and coworkers¹⁶⁻²². For our purposes the important feature of the life cycle (Fig. 1) is the formation of a 240S head precursor structure, the prohead (Fig. 2c and d), which contains the major coat protein—the product of gene 5—and a second major protein not found in mature phage, the product of gene 8. (Proheads also contain three minor protein species, the products of genes 1, 16 and 20, but these are not required for prohead formation^{17,18}.)

The prohead is the precursor head structure in the cutting and packaging of a mature length chromosome from the overlength replicating DNA. This process requires the products of genes 1, 2 and 3; if any of these proteins are missing (due to mutation), proheads and uncut DNA accumulate¹⁷. The immediate product of DNA encapsulation is unstable until acted on by the products of genes 10 and 26. If these proteins are missing the DNA comes back out, leaving empty heads, distinguishable from proheads by morphology and sedimentation. If cells are infected with *amber* mutants defective in gene 8, most of the coat protein remains unassembled, or assembles into aberrant aggregates¹⁸. Since P8 is needed for the assembly of the coat protein into phage, but is not found in phage, we termed it a 'scaffolding protein'¹⁸. The P22 prohead is similar in this respect to tau particles of phage T4 described by Kellenberger *et al.*²³ and Simon²⁴; Showe and Black⁹ and Laemmli and Favre²⁵ have shown that such particles contain proteins needed for morphogenesis but absent from the mature phage.

We show here that the isometric capsid of phage P22 does not self assemble. Roughly 200–300 molecules of the scaffolding protein catalyse the accurate polymerisation of the coat protein into a precursor shell containing both proteins. Upon DNA encapsulation, all the scaffolding protein molecules depart from the precursor shell. These then proceed to catalyse the polymerisation of newly synthesised coat protein molecules into another precursor shell.

Protein composition of proheads

Proheads are the earliest shell structures detectable in the head assembly pathway¹⁸. We isolated proheads in high concentration from infected cells, separated their proteins

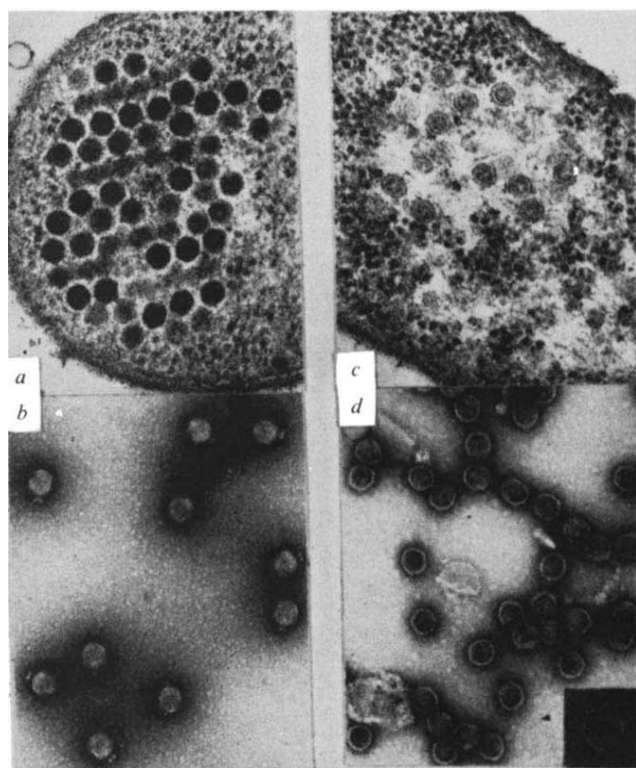


Fig. 2 Morphology of phage head structures. The upper panels are electron micrographs of ultrathin sections of P22 infected *Su⁻ DB21* (ref. 19) cells, prepared by the method of Simon²⁴. Each lower panel is an electron micrograph of particles (negatively stained) isolated from cells infected with the same phage as the panel above it. *a* and *b*, Normal phage particles; *c* and *d*, proheads from cells infected with phage carrying an amber mutation in gene 2. These structures contain both the coat protein and the scaffolding protein. The phage also carried the clear mutation *c₁*⁷ to prevent lysogeny and a lysis defective mutation in gene 13 (*amH101*)¹⁷. A more detailed account of the *in vivo* morphology of the various head-related structures formed during P22 infection will be presented later (Lenk, Casjens, Weeks and King, in preparation). The magnification of all panels is $\times 60,000$, and the magnification of the insert in panel (*d*) is $\times 140,000$.

by electrophoresis through SDS acrylamide gels, and determined mass ratios by staining with Coomassie brilliant blue and fast green. We first used cells infected with *amber* mutants defective in genes 1, 2 or 3, since proheads are the only particulate phage structures (240S) formed in them. Proheads from mutant-infected cells are qualitatively indistinguishable from the precursor proheads isolated from wild type-infected cells^{17,18}. Proheads were isolated from crude lysates by centrifugation through a sucrose-caesium chloride step gradient, and then further purified by centrifugation through a sucrose gradient, as described in the legend to Fig. 3. An electron micrograph of the resulting preparation is shown in Fig. 2*d*. Figure 3*h* (bottom right) shows the proteins of a 2⁻ prohead (proheads from cells infected with an *amber* mutant in gene 2) separated on an SDS gel and stained with Coomassie brilliant blue, with a densitometer tracing above. P8 is a major component. Proheads isolated from 1⁻, 2⁻, and 3⁻-infected cells were very similar in protein composition (except that the 1⁻ proheads lacked P1, as expected¹⁸).

The results of many gel analyses are summarised in Table 1. Taking 420 as the number of coat protein subunits¹⁷, and assuming equivalent binding of the stain to the two species of protein, we calculated that the proheads accumulating in 1⁻, 2⁻, and 3⁻-infected cells contain about 260 molecules of P8. In the lysates from which these particles were isolated, more than 90% of P5 and P8 molecules were in proheads.

To determine the ratio of scaffolding protein to coat protein in proheads from productive infected cells, we infected a culture with P22 and isolated proheads from samples lysed 22, 51, 90, and 109 min later. Figure 3*a-d* shows that P8 is a major component of these precursor structures. However, wild-type infected cells also contain empty heads, which lack P8 (ref. 18). These sediment at 170S and contaminate the 240S prohead fractions. From electron micrographs of negatively stained samples of the isolated wild type proheads, we estimated the contamination to be about 30%. Table 2 gives the number of P8 molecules in proheads isolated from productively infected cells, corrected for contamination by empty heads. The

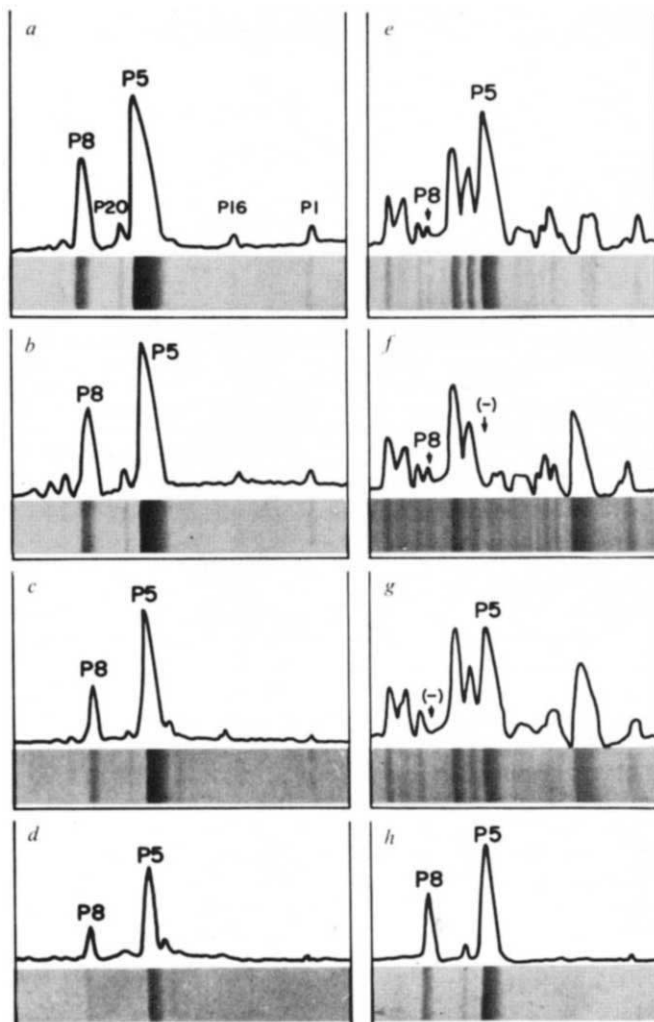


Fig. 3 SDS gel electrophoresis of the proteins in isolated proheads and in infected cells. P8 is a major protein of proheads, although it is much less predominant in whole lysates. Infected cells were prepared as described in Table 1 and proheads were purified as described in Tables 1 and 2. The proteins were separated by SDS gel electrophoresis³⁶ and the gel slabs stained with Coomassie brilliant blue²⁶. The stained gels are shown in the lower portion of each panel, with Joyce-Loebl microdensitometer tracings of the same gels above them. All the bands represent protein concentrations within the linear range of the staining reaction. Wild type proheads: *a*, 109 min; *b*, 80 min; *c*, 51 min; *d*, 22 min; *e*, Wild-type lysate, 80 min; *f*, 5⁻ lysate, 80 min; *g*, 8⁻ lysate, 80 min; *h*, 2⁻ proheads, 90 min. The high molecular weight regions of the gel are to the right. The band which is present in increased amounts in the 5⁻ and 8⁻ lysates is P9, the tail protein^{17,37}. Gene 9 is subject to a control system different from the other late proteins (Lew and Botstein, unpublished experiments): it is overproduced in cells infected with mutants blocked in DNA encapsulation. This phenomenon is independent of the overproduction of P8 described later in the text.

Table 1 Protein composition of proheads

	Stain	Molecules of P8 per 420 P5 molecules*
1 ⁻ Proheads	Coomassie blue	259 ± 16
	Fast green	245 ± 8
2 ⁻ Proheads	Coomassie blue	266 ± 16
	Fast green	not done
3 ⁻ Proheads	Coomassie blue	270 ± 7
	Fast green	251 ± 10
++ Proheads†	Coomassie blue	239 ± 25
	Fast green	252 ± 20

Proheads were prepared as follows: cultures of the su⁻ bacteria DB 21 (ref. 19) were grown to 2×10^8 cells per ml in broth²⁰ at 37° C, infected with P22 carrying the clear marker *c*₁⁷, a lysis-defective mutation in gene 13 (*amH101*) and an *amber* mutation in gene 1 (*amN10*), gene 2 (*amH200*), or gene 3 (*amN6*)¹⁷, at a multiplicity of five. After 90 min of aeration, cells were pelleted, resuspended in about 0.002 volumes of 0.02 M Tris-HCl, 10^{-3} M MgCl₂, pH 7.5, containing chloroform (to induce lysis) and pancreatic DNase (to reduce viscosity). The cell debris was removed by low speed centrifugation, and the clear, opalescent supernatant was layered on a 15–30% sucrose gradient and centrifuged at 20,000 r.p.m. for 2 h in an SW 27.1 rotor. Opalescent prohead bands were removed by piercing the side of the tube with a syringe. The proteins of the purified proheads were separated by SDS polyacrylamide slab gel electrophoresis^{35,36}. The resulting gels were stained with Coomassie brilliant blue²⁶ or fast green²⁷, and tracings of the coloured bands were made with a Joyce-Loebl microdensitometer. The ratio of copies of P8 to P5 was calculated from the areas under the peaks after correcting for the molecular weight differences. All measurements were made within the range in which stain density was proportional to the amount of sample applied to the gel.

* Average of a minimum of four polyacrylamide gel experiments. The errors are the calculated standard deviations.

† Corrected for the fraction (15–30% among 250–750 particles counted on electron micrographs) of contaminating empty heads (which contain no P8). The mutant prohead preparations contain no empty heads.

ratio of scaffolding protein to coat protein in these precursor proheads did not change significantly throughout infection, and was similar to the P8/P5 ratio in proheads isolated from 1⁻, 2⁻, and 3⁻ infected cells. The slightly higher P8/P5 ratio of the wild-type proheads may be due to overestimation of the contamination by empty heads.

Since the amino acid composition of these proteins is unknown, we do not know if P8 and P5 bind the same amount of dye per mg of protein. Published results indicate small differences in binding for different proteins^{26,27}. We therefore conclude that the prohead intermediates in phage P22 assembly contain 200–300 P8 scaffolding protein molecules for every 420 P5 coat protein molecules.

The ratio of P8 to P5 in proheads is quite different from the ratio of the two proteins in the total culture, as can be seen by comparing the lysate gel patterns of Fig. 3e with the prohead patterns. P22-infected cells accumulate very little P8 with respect to P5. The 5⁻ and 8⁻ mutant lysates (Fig. 3f,g) provide positive identification of the two proteins.

Figure 4 shows the rates of synthesis for the two proteins during infection, determined from the extent of incorporation of ¹⁴C-labelled amino acids during short pulses. At late times 15–20 copies of P5 coat protein were synthesised for every copy of P8 synthesised.

Botstein *et al.*¹⁷ showed that there was no detectable cleavage of late proteins in P22-infected cells. We have performed further pulse-chase experiments to look for proteolytic cleavage of P8, using shorter pulse times—

75 s at 30° C. Samples withdrawn from the culture at various times after chase were lysed immediately with chloroform, mixed with sodium dodecyl sulphate (SDS) sample buffer, and frozen before electrophoresis through an SDS gel slab. Recovery of radioactive P8 (weight of peak from densitometer tracing of autoradiogram) after the chase was as follows: 15 s, 56 mg; 2 min, 67 mg; 5 min, 73 mg; 9 min, 82 mg; 13 min, 73 mg; 18 min, 79 mg. We conclude that P8 is not cleaved to lower molecular weight during the phage life cycle. Since P8 is not in phage, and is not degraded, it must be removed from proheads intact.

P8 recycled during morphogenesis

If 200–300 molecules of P8 are required to make a prohead, and therefore a phage, and if each molecule of P8 were to take part in the formation of only one prohead, then Figs 3 and 4 indicate that too few molecules are made to account for the yield of viable phage particles. Pulse-chase experiments¹⁸ suggested that P8 is reused during infection; that is, after release from a prohead taking part in DNA encapsulation, the P8 molecules take part in further rounds of prohead assembly. If this is true, then P5 coat protein molecules should chase from proheads into phage, but the P8 scaffolding protein should remain with the prohead fraction, complexed with newly synthesised coat protein molecules.

To test this prediction, infected cells were exposed to radioactive amino acids continuously from 12 min after infection (which is before the onset of late protein synthesis) to 28 min after infection, so that all the molecules of P8 and P5 synthesised up to that time would be radioactive. At 28 min excess unlabelled amino acids were added to stop the uptake of labelled amino acids. At various times measured from the start of the chase (1, 2, 4, 7, 11, 16 and 22 min) samples of the culture were shaken with chloroform to lyse infected cells, and aliquots of the lysates were centrifuged through sucrose gradients, first to separate phage particles (500S), and then at higher speed to separate proheads and empty heads (240S and 170S). The radioactivity in the prohead and empty head peaks decreased with time after the chase, and the radioactivity in the phage peak increased, as expected¹⁸ (data not shown). To follow the fate of P5 and P8 molecules individually, we dissociated the prohead, empty head, and phage-containing sucrose gradient fractions in hot SDS, and electrophoresed the samples through an SDS gel slab. The resulting autoradiograms displayed the expected band patterns¹⁸: proheads contained P5 and P8 as the dominant bands, plus much smaller amounts of radioactive P1, P16 and P20. In comparison the phage lacked the P8 band but displayed tail protein (P9) and P26 and PX. The empty heads were similar to the proheads except they also lacked P8. Figure 5 shows that labelled coat protein chased from proheads and empty heads into phage, while labelled scaffolding protein remained associated with the prohead fraction. Since the ratio of P5 and P8 in proheads does not change during infection (Table 2) the radioactive P8 must have associated with nonradioactive coat protein

Table 2 Number of molecules of P8 in head structures isolated at various times after infection

	22 min	51 min	80 min	109 min
Proheads	295 ± 30	290 ± 25	315 ± 30	300 ± 25
Empty heads	< 10	< 10	< 10	< 10
Phage	< 10	< 5	< 2	< 2

Errors represent the limits of at least two determinations. Particles were purified and values for P8 determined as described in the legend of Table 1.

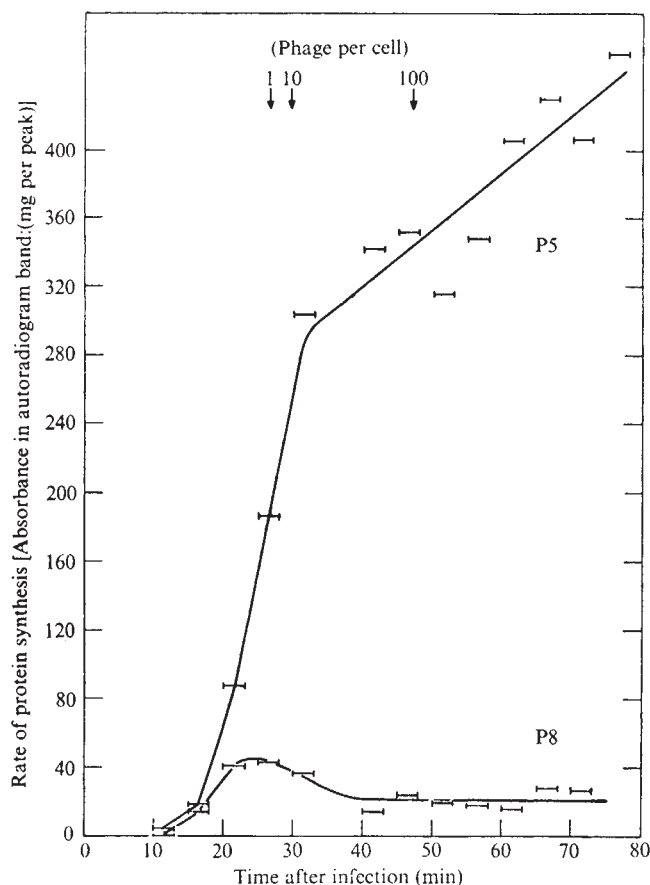


Fig. 4 Rate of P8 and P5 synthesis in phage-infected cells. *S. typhimurium* D21 was grown in M9 medium¹⁷ at 37° C to 2×10^8 cells per ml and infected with five $c_1/3^-$ phage per cell. At the times shown, samples of the culture were incubated with ^{14}C -labelled amino acid mixture to give a final concentration of $0.2 \mu\text{Ci ml}^{-1}$. Three minutes later incorporation was stopped by addition of excess casamino acids, and after a further 1.5 min incubation, the cells were chilled in ice and then centrifuged at 4,000 r.p.m. for 10 min. The resulting pellet was resuspended in 1/4 the original volume and lysed by shaking vigorously with chloroform. Samples were heated in SDS sample buffer and the proteins separated by electrophoresis through a 12% SDS polyacrylamide slab gel^{35,36}. Densitometer tracings of appropriately exposed autoradiograms were prepared³⁸, and the peaks representing the bands of radioactive P8 and P5 cut out and weighed. Experiments performed at 30° C gave similar results, as did experiments in which cells were lysed immediately after the chase of the radioactive amino acids.

synthesised after the chase. The simplest interpretation of these results is that on encapsulation of DNA by the prohead, all the P8 molecules dissociate from the complex, and rapidly reassociate with more coat protein to form further proheads. (These proheads would also be expected to contain newly synthesised unlabelled P8, which would not have been detected in the experiment.)

Suppose a prohead is formed of both previously synthesised and newly synthesised scaffolding protein, but only newly synthesised coat protein; then after short pulses of label, the ratio of labelled P8 to labelled P5 in proheads should decrease for pulses given later and later during infection, since the pool of unlabelled recycling P8 will increase with respect to the pulse labelled P8. To test this prediction infected cells were exposed to 3-min pulses of radioactive amino acids at various times after infection. Two and one half minutes after the chase of radioactivity, samples were lysed and centrifuged through sucrose gradients to separate proheads. This was sufficient time for much of the pulse labelled P5 and P8 to form proheads, but not long enough for substantial label to appear

in phage¹⁸. The prohead proteins were separated by SDS gel electrophoresis and autoradiograms prepared and tracings made from them. The molar ratio of P8 to P5 radioactivity in pulse labelled proheads was as follows: 33 min, 21%; 44 min, 9.9%; 51 min, 7.8%; 60 min, 5.2%. The ratio of labelled P8 to P5 in proheads clearly decreased as the infection progressed. Since the total protein composition of these particles was not different (Table 2), we ascribe the decrease to the presence of unlabelled recycling P8 synthesised before the pulse period.

How many rounds of prohead assembly does each P8 molecule participate in, on the average? To answer this question we measured the total amount of P5 and P8 in the whole lysate by Coomassie brilliant blue staining of SDS gels, and also measured the P5 and P8 in the proheads, empty heads, and phage fractions isolated from the lysate. Given that it takes about 250 molecules of P8 for the incorporation of 420 molecules of P5 into phage, we calculated how many times the P8 in the culture at the time of lysis would have had to function, to account for the amount of coat protein assembled into particles (phage + empty heads + proheads) present in the culture at the time. As Fig 6 shows, this number increases during infection and is about 5 at the time the cells would have lysed (45 min) if the phage were not carrying a lysis delay mutation.

Since this is a somewhat unusual catalytic protein, in which the formation of the product requires the concerted reaction of more than two hundred catalyst molecules, we have not expressed the data in Fig. 6 as an enzymatic turnover number. The value corresponding to that is about 0.2 phage per minute per 250 P8 molecules at 37° C.

Prohead assembly

The experiments described here show that about 250 molecules of the 42,000 molecular weight gene 8 scaffolding protein catalyse the assembly of about 420 molecules of the 55,000 molecular weight gene 5 coat protein into a precursor prohead shell containing both proteins. The scaffolding protein then exits from the prohead in concert with the condensation of the phage DNA, yielding a packaged chromosome and free scaffolding protein, which then repeats the cycle. Proheads could be described as giant enzyme-substrate complexes; no scaffolding protein is detectable in virus particles containing DNA, the product of the reaction.

That proper shell formation depends on the participation of the scaffolding protein is shown most clearly by the analysis of gene 8 mutants: cells infected with amber mutants defective in gene 8 synthesise normal amounts of coat protein (Fig. 3f) but this protein does not assemble efficiently into closed shells^{17,18}. Instead, aberrant aggregates are formed; among these are shells with smaller diameter than phage, and larger 'spiral' structures. This latter class looks like a shell which has failed to join its growing edges, and then kept adding subunits at the exposed edge. Nested shells have also been seen. We have performed pulse-chase experiments with δ^- -infected cells which show that P5 assembles into aberrant structure less than 1/5 as efficiently as the P5 in wild-type infected cells assembles into proheads. These experiments suggest that the scaffolding protein increases both the rate and accuracy of shell formation.

What is the structure of the prohead complex? The electron microscopic appearance of proheads in both negatively stained specimens and ultrathin sections of infected cells shows organised internal structure (Fig. 2c, d). The images suggest either a double shell structure, or a ball within a shell. Since the outer shell has dimensions similar to the mature phage, and also represents more mass than the inner material, we presume that the outer

shell is made of coat protein, and the inner material represents the scaffolding protein. The two proteins could be intimately associated as a single thick shell, and the appearance in thin sections could be due to an artefact of

fixation or staining. Since the capsid of the mature phage is most likely a lattice with icosahedral symmetry², it seems probable that the prohead also represents an icosahedral type surface lattice. Much less clear is the symmetry expected of the scaffolding protein organisation.

How does the scaffolding protein control the assembly of the coat protein? The scaffolding protein itself might assemble into a large complex which serves as a template or 'assembly core' for coat protein polymerisation, as suggested^{9,23} for phage T4. If that were the case we would expect to find such complexes in cells infected with coat protein amber mutants. We find, however, that all the scaffolding protein in 5⁻ lysates sediments as free subunits (<4S). We have also been unable to see organised structures in thin sections of 5⁻ infected cells.

These observations suggest that the scaffolding protein complexes not with itself initially, but with coat protein, and that a coat protein/scaffolding protein multimer is the functional unit in shell formation. Presumably in the absence of the scaffolding protein, the bond angles between coat protein molecules are not specified rigidly enough to ensure that over a 1,900 Å circumference the edges will join properly. Multimers of coat protein and scaffolding protein would be expected to have much larger reactive surfaces and thus be able to specify bond angles more accurately.

Figure 4 shows that P8 is synthesised at a low level in wild-type infected cells. We have also measured the synthesis of late proteins in cells infected with amber mutants in each of the P22 late genes. The pattern of P8 and P5 synthesis is the same as wild type in cells infected with mutants which are capable of DNA packaging (16⁻, 20⁻, 10⁻, 26⁻ and 9⁻). But, cells infected with mutants defective in genes, 1, 2, or 3 overproduce P8 4–8-fold. In these cells P8 is trapped in the prohead complexes which accumulate and is not recycled. The overproduction is not due simply to the large pool of unpackaged DNA that accumulates, since 5⁻ infected cells do not overproduce P8. Various experiments on the rate of P8 synthesis in mutant infected cells (unpublished experiments) indicate that the level of P8 synthesis is a function of the concentration of unassembled P8. Its synthesis is thus self-regulated, similar to the *Salmonella* histidine repressor²⁸, threonine deaminase²⁹ and T4 DNA polymerase³⁰. In the case of phage head assembly, this provides a feedback loop between DNA packaging and protein synthesis; if encapsulation is proceeding efficiently, P8 is released to form further proheads, and depresses its own further synthesis.

Since P8 is a major component of proheads, its catalytic function saves the cell considerable protein synthetic capacity; the economy is even greater when the synthesis is regulated so that the protein is produced at a rate just sufficient for optimal assembly.

P8 and DNA condensation

Although we have focused on its scaffolding function, the DNA condensation function of P8 might be of equal biological importance. Since all phage head structures containing DNA lack P8, the 200–250 molecules of P8 must exit from the prohead before or during DNA encapsulation. This unusual exchange reaction requires the products of genes 1, 2, and 3, since if any one of them is missing, proheads and unpackaged DNA accumulate¹⁷. P2 and P3 are not associated with proheads or phage; we suspect that they interact with precursor DNA, perhaps forming a binding site for proheads. The actual condensation of the DNA within the head might be directly coupled with the exit of P8. According to Laemmli's model for DNA packaging²³, for example, the exit of P8 might expose charged sites within the head which serve to collapse the DNA. The scaffolding protein might exit by

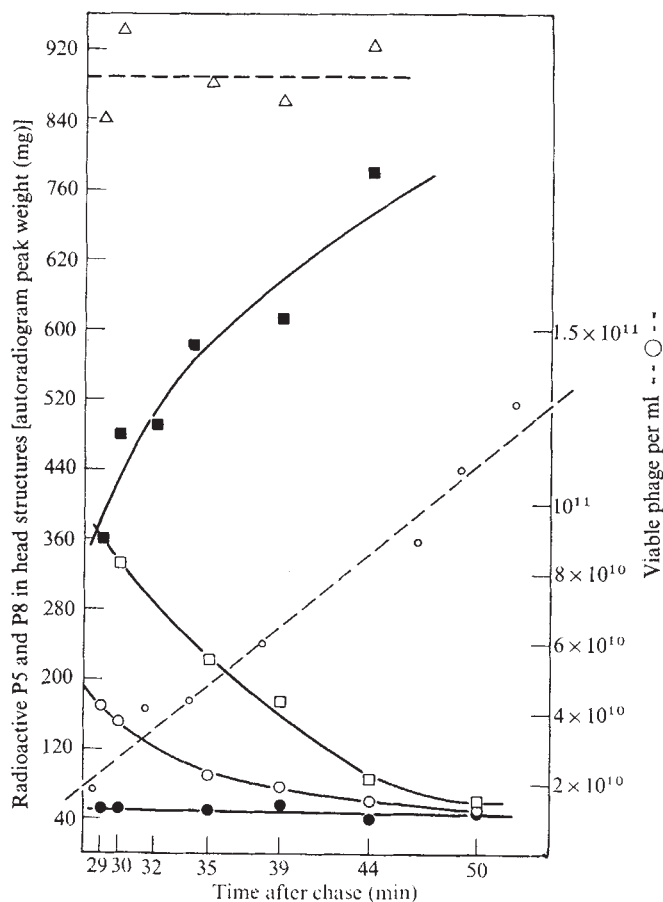


Fig. 5 Continued association of labelled scaffolding protein with proheads during phage growth. This figure summarises the fate of labelled coat and scaffolding protein after a chase of radioactivity which had been present continuously from 12 to 28 min after infection. The amount of labelled P5 in the phage fraction (■) increases after the chase while the amount of labelled P5 in the prohead (○) and empty head fractions (□) decreases with time after the chase. However, all of the labelled P8 associated with proheads (●) at the time of the chase remains associated with proheads even though these structures are serving as intermediates in assembly and the P5 associated with them is converted to phage. That viable phage are continuously forming is shown by the dashed line. The open triangles show the sum of the labelled P5 from the various fractions. Though not shown in the figure, the minor proteins present in proheads P1, P16 and P20 also chased from the prohead fraction into the phage fraction during the chase. The experiment was performed as follows: D21 was grown in M9 medium¹⁷ at 30°C to 2×10^8 cells per ml and infected with 10^6 ϕ 13⁻ phage per cell. At 12, 16, 20 and 24 min after infection, 2.5 μ Ci of 14 C-labelled amino acid mixture was added per ml of culture (giving a final concentration of 10 μ Ci ml⁻¹). At 28 min excess casamino acids were added and at 1, 2, 4, 7, 11, 16 and 22 min thereafter, samples were lysed by shaking vigorously with chloroform. Incorporation of radioactivity into trichloroacetic acid insoluble material was linear during the labelling period. For separation of phage, 0.25 ml aliquots were centrifuged through 5–20% sucrose gradients in 0.01 M Tris buffer, with a shelf of 0.3 ml of CsCl, $\rho = 1.6$ g cm⁻³. Centrifugation was for 20 min at 20,000 r.p.m., 20°C. For separation of proheads and empty heads (a and c) 0.1 ml aliquots were centrifuged for 30 min at 30,000 r.p.m., 20°C. Radioactivity was determined by mixing aliquots of the gradient fractions with Triton X-100/toluene based scintillation fluid and counting in a scintillation counter. Particles from the sucrose gradient separations were dissociated in SDS sample buffer and electrophoresed through a 10% SDS polyacrylamide slab gel and the radioactive bands determined by autoradiography. Densitometer tracings of the autoradiograms were made and the peaks cut out and weighed.

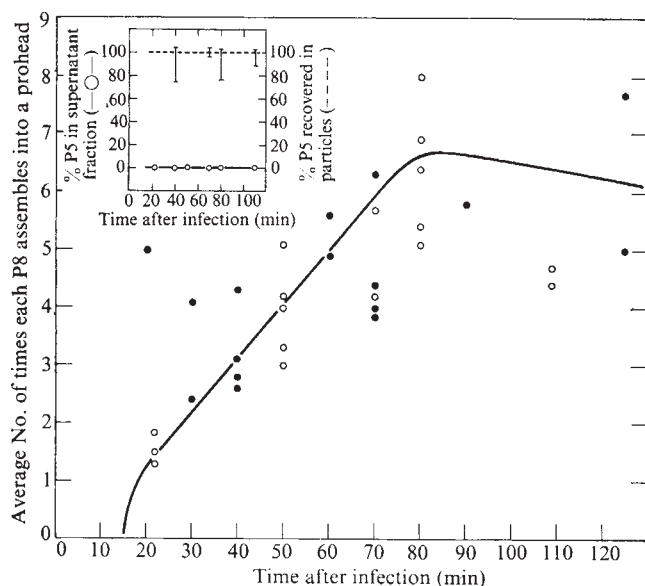


Fig. 6 Recycling of scaffolding protein molecules. The graph shows the average number of rounds of prohead assembly that each scaffolding molecule has taken part in during phage growth. The calculation was as follows:

$$\text{number of times P8 reused} = \frac{(P8/P5)_{\text{prohead}}}{(P8/P5.F)_{\text{cells}}}$$

where F is the fraction of the total P5 molecules in the cell which is in phage or phage precursor structures. F was found to be very near 1.0 (see below). The open and closed circles represent two independent experiments. *S. typhimurium* DB21 was grown in broth to 2×10^8 cells per ml and infected with P22 $c_1/3^-$ at a multiplicity of 10. At the times indicated samples of the infected cells were pelleted, resuspended in 0.1 volume of electrophoresis buffer, heated to 100°C for 2 min, and the proteins were separated by SDS slab gel electrophoresis (see Fig. 3). The protein bands were quantitated by tracing with a Joyce-Loebl microdensitometer, and the areas under the P8 and P5 peaks measured. At various times after the infection described above, samples were also taken for analysis of the phage-specific structures present. The infected cells were concentrated by centrifugation, lysed with CHCl_3 , layered on the top of a sucrose gradient with a high density (1.6 g cm^{-3}) CsCl cushion, and centrifuged for 45 min at 45,000 r.p.m. in a Beckman SW 50.1 rotor. The fraction containing all the particles (phage, proheads and empty heads), that is, the top of the CsCl cushion, and the supernatant (top 1.5 ml) fraction were removed with a syringe, and the proteins separated and quantitated as above. The insert in the figure above shows the results of such an analysis normalised to the total amount of P5 present.

rearrangement of the coat protein shell over its entire surface, or through a single channel. If the exit of P8 molecules is directly coupled to the entry of DNA, then the scaffolding protein catalyses not only shell formation, but also DNA condensation, since each molecule takes part in as many cycles of DNA packaging as it does of prohead formation.

Since the P22 chromosome is packaged by a 'headful cutting' mechanism^{16,17,31}, the length of the chromosome must be determined by the size of the head. Thus the scaffolding protein, in specifying accurately the dimensions of the prohead shell, is specifying the length of the virus chromosome.

Proheads are similar in morphology to the T4 tau particles described by Kellenberger *et al.*²³; Simon²⁴ and Laemmli³² showed that these are precursors to phage, and Showe and Black⁹ demonstrated that the inner material is probably an assembly core composed of the product of T4 gene 22 product and internal proteins. The 22 product and internal protein III are cleaved down to fragments during DNA packaging²⁵. Thus, the P22 scaffolding protein and T4 assembly core are formally equivalent; in the former case the assembly core is removed by proteolytic cleavage during DNA packaging and in the latter case the protein

is removed intact and then recycles. Bacteriophages T7 and T3 may be assembled similarly to P22 since during infection a top component occurs which contains a protein absent from phage^{33,34}. This protein is not cleaved during assembly, and is specified by a gene which maps adjacent to the coat protein, just as in P22.

Catalysis of morphogenesis

The first case in which a protein was shown to catalyse a step in morphogenesis was the attachment of T4 tail fibres to particles by gene 63 product (Wood and Heninger⁶). The gene 63 product is typical of an enzyme in that a stable complex with the two substrates could not be isolated. We think the scaffolding protein represents a new kind of catalytic function: (a) hundreds of protein molecules are necessary for the formation of one product of the reaction; (b) the catalyst molecules become a stable component of the immediate product of the reaction—in essence the scaffolding protein specifies the correct dimensions of the coat protein shell by temporarily becoming part of the shell; (c) the dissociation of the complex is a very complicated reaction requiring at least three other proteins, plus the encapsulation of a viral chromosome.

Perhaps most important, these results show clearly that proteins critical for assembly may not be found as part of the mature structure^{6-9,25}. For example, the proteins which condense DNA into chromosomes in higher organisms may not be found in the mature chromosome. We would also like to emphasise that although a major component of a viral precursor, the P22 scaffolding protein — because of its ability to recycle — is only a minor component of phage-infected cells. We suspect that many structure formation processes will turn out to proceed by a similar combination of catalytic with self-assembly mechanisms.

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Operator and promoter are separate in the tryptophan operon in *Salmonella typhimurium*

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Studies of the trp mRNA made by Salmonella typhimurium strains carrying trpOA and trpOAB multisite mutations have shown that these are deletions. They do not extend into the trp promoter (trpP1) as shown by their ability to recombine with trpP4 mutation. Thus the trp operator (trpO) and the trp promoter are separable and arranged in the order P1-O-A.

In a preliminary report¹ we presented evidence that in the *trp* operon of *Salmonella typhimurium* the promoter (*trpP1*) and operator (*trpO*) were separable and arranged in the order *P1-O* in relation to the first structural gene *trpA* (Fig. 1). Part of this evidence was the observation that we were unable to find *supX-trpO* deletions but easily obtained multisite mutations with the characteristics of deletions extending from *trpA* to *trpO* and leaving *trpP1* intact. We did not, however, exclude the possibilities that these multisite mutations were (a) insertions of extraneous genetic material^{2,3} containing a functional promoter, or (b) deletions extending beyond *trpP1* and joining the remainder of the *trp* operon to an outside promoter. We report here that our multisite mutations are indeed *trpA-trpO* deletions which do not extend fully into *trpP1*, thus strengthening our earlier conclusions about the order and separability of *trpP1* and *trpO*.

The isolation of our multisite mutations was based on the observation that strains carrying strong polar mutations in *trpA* or in *trpB*-region I (Fig. 1) grow very slowly on anthranilic acid supplement since they have very low levels of PRPP-phosphoribosyl transferase (PRT (EC 2.4.2.18) activity⁴ Secondary mutants of such strains selected for improved utilisation of anthranilic acid, a substrate of PRT, carry deletions of various portions of the *trpA* gene and, depending on the mutants, *trpB*-region I (refs 4,5). Representatives of this class are *trpAB688* and *trpAB674* in Fig. 1. By selecting simultaneously for normal growth on anthranilic acid supplement and resistance to 5-methyltryptophan (SMT), an analogue of tryptophan able to act as a co-repressor⁵, we obtained exclusively multisite mutations extending from *trpA* to *trpO* (ref. 1). These are represented by *trpOA526*, *trpOAB673* and *trpOAB1349* in Fig. 1. Since these presumptive deletions correct for a polar effect by increasing the level of activity of a growth limiting enzyme

(PRT), they should end 'in phase'. By transducing the mutant *cysB403* and the double mutant *cysB403 ΔtrpPOABEDC167* with lysates grown on each multisite mutant and plating on tryptophan supplemented minimal medium, we determined that all multisite mutations co-transduced with *cysB* at the same frequency as the wild type *trp* operon and were not translocations of the distal segment of the operon to the vicinity of some other promoter on the *S. typhimurium* chromosome.

We have been able to look at the *trp* transcripts of our *S. typhimurium* mutants by taking advantage of the observation of Denney and Yanofsky⁷ that *S. typhimurium*

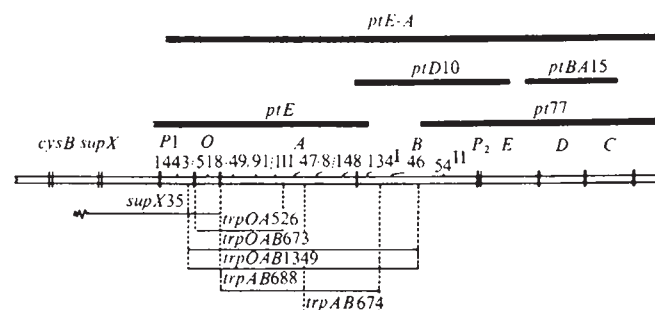


Fig. 1 Genetic map of the *trp* operon of *Salmonella typhimurium* (open bar, centre) showing the segments covered by the various multisite mutations used in this investigation (below bar) and the corresponding portions of the *trp* operon of *Escherichia coli* carried by transducing Ø80-pt phages used in hybridisation experiments (closed bars above map). The *trp* operons of both *E. coli* and *S. typhimurium* consist of five closely linked structural genes under tryptophan control^{10,11}. The order of these genes is the same in both organisms but the nomenclature is different: that is the first structural gene, which codes for the anthranilate synthetase (AS) component of the AS-PRT complex, is designated *trpE* in *E. coli* and *trpA* in *S. typhimurium*^{10,11}. The numbers above the bar represent point mutations of the early portion of the operon and are shown in their map order^{1,3,6}. P1 represents the main *trp* promoter and P2 is the low-efficiency internal promoter-like element^{21,27}. The map indicates both regions of *trpB*, the gene for the anthranilate-PRPP-phosphoribosyl transferase (PRT) component of the AS-PRT enzyme complex: region I codes for the glutamine transferase activity of this component, and region II for the PRT activity proper²⁸. The isolation of the multisite mutations is described in the text. The isolation of *supX35* was described by Margolin and Bauerle²⁷.

Table 1 Levels of *trp* mRNA and tryptophan synthetase (TS) in mutant strains

Strain	Genotype	Repressed	TSase relative specific activity	Derepressed					TSase relative specific activity
		³ H-RNA (% of input c.p.m.) hybridised to DNA of		³ H-RNA (% of input c.p.m.) hybridised to DNA of					
		<i>pt E-A</i>		<i>ptE</i>	<i>pt77</i>	<i>ptBA15</i>	<i>ptD10</i>	<i>pt E-A</i>	
—	<i>trpD9</i>	0.008	0.5	0.416	0.735	0.33	0.39	1.215	119
SO-160	<i>trp0A526</i>	1.022	90	0.012	0.554	0.39	0.41	1.048	85
SO-441	<i>trp0AB1349</i>	0.249	6	0.011	0.264	0.20	0.21	0.429	3.2
SO-584	<i>trp0AB673</i>	0.367	6	0.016	0.441	0.23	0.25	0.470	3.2
SO-631	<i>trpAB674</i>	0.010	1.2	0.080	0.592	0.47	0.49	1.160	118
SO-645	<i>trpAB688</i>	0.005	1.25	0.010	0.560	0.46	0.49	1.040	123

The nomenclature used for the bacterial strains follows the recommendations of Demerec *et al.*¹⁵. For pulse labelling experiments cells (8×10^8 ml⁻¹) grown at 37° C in L broth were collected, washed twice with cold Vogel and Bonner's minimal medium¹⁶ supplemented with 1% glucose and 0.25% casamino acids and resuspended in the same medium at an approximate concentration of 2×10^8 cells per ml. Tryptophan (50 µg ml⁻¹) was added (repression conditions) or omitted (derepression conditions) as needed. A 10 ml sample of the cell suspension was incubated at 30° C on a shaker for 10 min and then pulse labelled for 2 min with 10 µCi ml⁻¹ carrier-free ³H-5, 6-uridine (37 Ci mmol⁻¹). Labelling was stopped and the cells killed by pouring the culture immediately on to an equal volume of crushed frozen medium consisting of 0.01 M Tris-HCl buffer (pH 7.3), 5 mM MgCl₂, 10m M Na₂S₂O₈, 400 µg ml⁻¹ chloramphenicol, and 20% sucrose. RNA was prepared by the phenol extraction method of Okamoto *et al.*¹⁷ except that the treatment with 0.5% sodium dodecylsulphate (SDS) and phenol was carried out at 4° C. The RNA was precipitated with ethanol, dissolved in 0.5 M KCl, 10m M Tris-HCl buffer (pH 7.3) and filtered through a Millipore filter (0.45 µm pore size). After being further precipitated and redissolved by the same procedure, this purified ³H-RNA was hybridised with denatured DNA of phage φ80 and the transducing phages described in Fig. 1. 30–50 µg of ³H-RNA were used for each assay. Bacteriophage DNA was prepared essentially as described by Morse and Yanofsky¹⁸, with some modifications, heat denatured at 100° C for 20 min, rapidly cooled and bound to Millipore filters of 0.45 µm pore size (10 µg per filter) as described by Gillespie and Spiegelman¹⁹ and modified by Morse and Yanofsky¹⁸. Hybridisation of ³H-RNA to the filter-bound DNA was carried out in 0.4 ml volumes of 1 × SSC (0.15 M NaCl, 0.015 M trisodium citrate). The reaction mixtures were incubated for 18 h at 65° C and cooled slowly to below 40° C. The filters were then washed twice with cold 2 × SSC, incubated for 30 min at 60° C in this buffer and rinsed twice with cold 2 × SSC to reduce nonspecific binding, dried and the radioactivity determined in a liquid scintillation counter using a toluene base scintillation mixture. No RNase treatment was used since this decreased considerably the number of counts hybridised in heterologous mRNA–DNA mixtures⁷. The results given have been corrected by subtracting the counts retained on control φ80 DNA filters (about 20 c.p.m.). Total radioactivity of ³H-uridine incorporated into RNA (total ³H-RNA) was measured as counts incorporated into cold TCA-precipitable material ($\sim 10^6$ c.p.m.). Tryptophan synthetase (TS) was assayed according to the procedure of Smith and Yanofsky²⁰. The β component of this enzyme was assayed in all strains except *trpD9*, known to lack it. For this strain the α component was assayed. Since both components of the tryptophan synthetase complex are repressed and derepressed in a coordinate fashion^{21,22}, the level of either of them can be used as an index of the level of expression of the *trp* operon. Cells for the enzyme assays were grown in minimal medium¹⁶ containing 0.5% glucose and 2 µg ml⁻¹ casamino acids and supplemented with different amounts of L-tryptophan: 50 µg ml⁻¹ for repressing growth conditions, and 5 µg ml⁻¹ for derepressing growth conditions. Protein was determined by the method of Lowry *et al.*²³. One unit of specific activity is defined as the utilisation of 0.1 µmol of substrate (indole) per mg protein at 37° C in 20 min²⁰. Specific activities are relative to those of repressed wild type (specific activity = 0.4) taken as unity.

trp mRNA can hybridise to *E. coli trp* DNA carried by transducing φ80 *pt* phages if certain modifications are introduced into the standard hybridisation procedure. Under our conditions, hybridisation in the heterologous *S. typhimurium* mRNA–*E. coli* DNA system was about 67–75% of that in the homologous *E. coli*–*E. coli* system in good agreement with the results of Denney and Yanofsky. Table 1 summarises some properties of our multisite mutants and the control strain *trpD9*, which carries a nonpolar mutation in the *trpD* gene. All the strains in which the multisite mutation extends into the operator make tryptophan synthetase (TSase, EC 4.2.1.20) and *trp* mRNA constitutively, while in those which have an intact operator this enzyme and *trp* mRNA are under tryptophan control. Table 1 also shows that the multisite mutants, (with the exception of *trpAB674*), have no detectable mRNA hybridising to the DNA of the phage *ptE*, but they do have detectable levels of mRNA hybridising to *pt77*, *ptBA15* and *ptD10* as well as *ptEA* DNA. Since *ptE* carries only the

DNA of the first structural gene of the operon, these are the results we would predict if the multisite mutations were deletions of the segment indicated in the genetic map (Fig. 1). We would anticipate that *trpOΔ526* and *trpAB674* might contain some *trp* mRNA hybridisable to *ptE* DNA, but found a very small amount of it only in *trpAB674*. We do not understand this observation yet, but it could be related to the decreased homology between mRNA and DNA in our heterologous system. In any case, there is a distinct difference between *trpD9* and our multisite mutants in their content of *trp* mRNA hybridisable to *ptE* DNA.

The *trp* mRNA of *E. coli* is about 6,700 nucleotides long⁸ and has a sedimentation coefficient of 33S in 5–30% linear sucrose gradients⁹. Since the *trp* operons of *S. typhimurium* and *E. coli* are identical in overall organisation and the sizes of their protein products^{10,11}, we expect that the *trp* mRNA of *S. typhimurium* will have the same sedimentation coefficient as that of *E. coli* when centrifuged under the

Table 2 Transcription times (min) of various segments of the *trp* operon after the addition of rifampicin

Strain	Genotype	Transcription times (min) of segments hybridised to DNA of				<i>trp</i> mRNA			
		<i>ptE</i>	<i>ptD10</i>	<i>ptBA15</i>	<i>ptEA</i>	Estimated no. of nucleotides	Molecular weight	Predicted S values	Observed S value
—	<i>trpD9</i>	1.0	2.25	4.7	4.7	6700	2×10^6	33	33
SO-160	<i>trpOΔ526</i>	0	1.16	4.0	4.0	5800	1.7×10^6	29	30
SO-441	<i>trpOΔB1349</i>	0	1.0	3.4	3.4	4800	1.4×10^6	26	25
SO-584	<i>trpOΔB673</i>	0	1.0	3.5	3.5	5100	1.5×10^6	27	23
SO-631	<i>trpAB674</i>	0.7	1.8	4.0	4.0	5800	1.7×10^6	29	27
SO-645	<i>trpAB688</i>	0	1.5	3.4	3.4	4800	1.4×10^6	26	24

The experiment was carried out as described in the legend to Fig. 3. Estimated sizes for the *trp* mRNA are based on transcription time. A value of zero indicates that no detectable ³H-mRNA was found to hybridise to a particular *pt* DNA. The predicted S values were obtained from a standard curve of S against molecular weight of various species of RNA based on figures obtained from the literature. The observed S values were determined by the procedure described in the legend to Fig. 2.

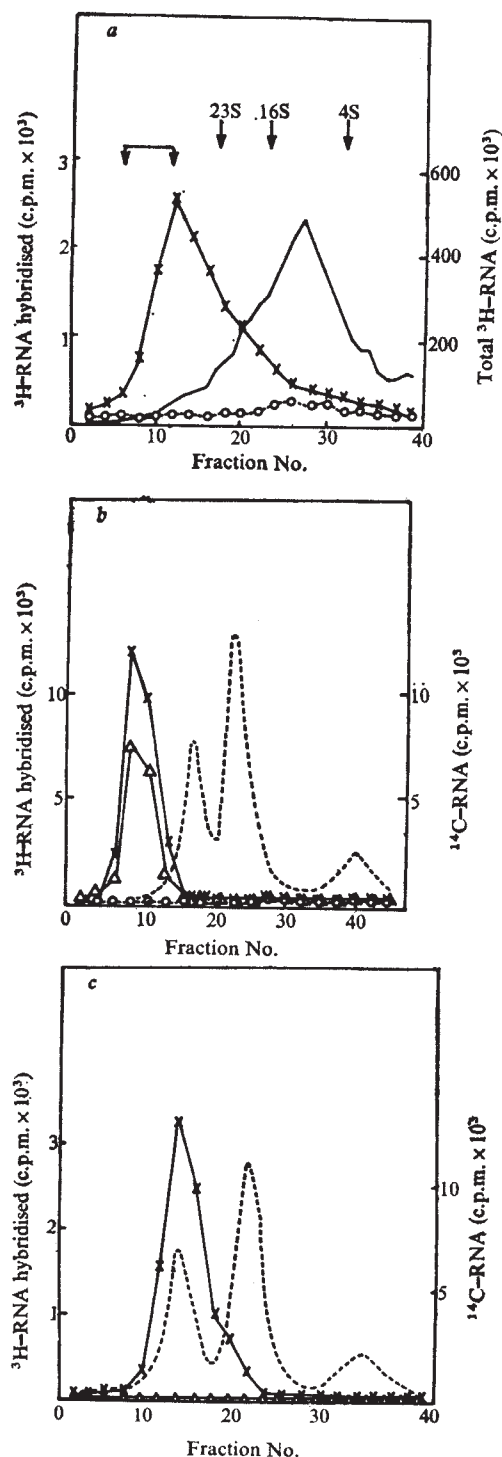


Fig. 2 Sedimentation (a) and resedimentation (b and c) profiles of *trp* mRNA of the mutants *trpD9* and SO-584. a, The procedures used for pulse labelling, killing the cells and isolating ^3H -RNA are described in the legend to Table 1. Cells were labelled under derepression conditions (that is in the absence of tryptophan). The purified ^3H -RNA preparation (300–500 μg RNA, 4×10^6 c.p.m.) was sedimented on a 5–30% linear sucrose gradient in 0.01 M Tris-HCl buffer (pH 7.3) and 0.05M KCl for 6 h at 36,000 r.p.m. in a SW39 swinging bucket rotor of a L2-65B Spinco refrigerated ultracentrifuge. Fractions of 10–16 drops were collected, and after adjusting the concentration of KCl to 0.5M, two neighbouring RNA fractions were pooled and 1/5 of each pooled fraction used for determination of the A_{260} , total ^3H -RNA counts and the hybridisation assay while the remainder was kept for resedimentation. The hybridisation procedure is described in the legend to Table 1. X, counts retained on *ptEA*-DNA filters; O, counts retained on ϕ 80-DNA filters; —, counts incorporated into cold TCA-precipitable material (total RNA). b, The bottom fractions containing the heaviest *trp* mRNA species (indi-

same conditions. This is indeed the case, as shown in Fig. 2, for the *trp* mRNA of *trpD9*. The mRNA molecule at the peak of the resedimentation profile (Fig. 2b) sedimented at about 33S. This *trp* mRNA could hybridise to *ptE* as well as *pt77* DNA, as expected from a normal polycistronic *trp* mRNA. Figure 2c shows the resedimentation profile SO-584 (genotype *trpOAB673*). In contrast to *trpD9* the largest *trp* mRNA of this mutant sediments at 23S and does not hybridise to *ptE*, indicating that it is a transcript of only the distal portion of the operon. The *trp* mRNAs of all our multisite mutants had smaller sedimentation coefficients than that of the control (Table 2), as expected if they contain deletions in the *trp* operon.

Measurements of transcription time further support the idea that our multisite mutations are deletions of the proximal portion of the *trp* operon. Rifampicin inhibits the initiation of transcription but does not interfere with transcription which is in progress. A plot of the rate of ^3H -uridine incorporation after addition of rifampicin is linear¹² in agreement with the assumptions that (a) the distribution of RNA polymerase molecules transcribing the operon under derepression conditions is random, and (b) all polymerase molecules move at a constant rate. Transcription time can then be obtained by extrapolation of the line expressing the rate of ^3H -uridine incorporation to the time axis¹². As Fig. 3 shows, it takes about 4.66 min to transcribe the *trp* operon into *trpD9*. For a mRNA which is 6,700 nucleotides long this corresponds to a transcription rate of 24 nucleotides per s, quite close to that calculated for *E. coli* (25–28 nucleotides per s)¹². The figure also shows that the transcription time for the small deletions *trpOAS26* and *trpAB674* is shorter (4 min) than that for *trpD9*, and that for the larger deletions *trpOAB637*, *trpOAB1349* and *trpAB688* is shorter still (about 3.5 min). Estimates of the size of the *trp* mRNA of each mutant based on these data are given in Table 2, and predicted S values for the respective *trp* mRNAs are compared with the observed ones. The agreement between the estimated and the observed values is reasonably good.

If ^3H -RNA is hybridised to the DNA of *pt* phages carrying different segments of the *trp* operon, such as *ptE*, *ptD10* and *ptBA15* (Fig. 1) we can obtain additional evidence that the deletions are limited to the beginning (*trpOAB*) portion of the operon. Thus, if the first structural gene or parts thereof are missing, transcription of the second structural gene should end earlier in the mutant strains than in the control. Results from these experiments are shown in Table 2. In *trpD9* synthesis of mRNA for the first gene (portion hybridisable to *ptE* DNA) is completed in 1 min, that for the second gene and early portion of the third (portion hybridisable to *ptD10* DNA) is completed in 2.25 min, and the mRNA for most distal portion of the operon (hybridisable to *ptBA15*) is completed in 4.7 min,

cated by arrows in a) were combined and mixed with ^{14}C -rRNA prepared as described below. All the RNA was then precipitated with ethanol, resuspended in H_2O , layered on a 5–30% sucrose gradient and centrifuged as described above. After centrifugation, two neighbouring fractions were combined and hybridised with various DNAs. The ^{14}C -rRNA was prepared by growing a strain carrying a deletion of the entire *trp* operon (*trpPOABEDC167*) under repression conditions (see legend to Table 1) and labelling with 50 μC of ^{14}C -uridine (52.7 mCi mmol⁻¹) for 3 h at 37° C. The cells were collected, washed twice and resuspended in 10 ml of the same medium. After culture for 3 further hours, they were killed and the ^{14}C -RNA extracted by the procedures described in the legend to Table 1. X, counts retained on *pt77*-DNA filters; Δ , counts retained on *ptE*-DNA filters; O, counts retained on ϕ 80-DNA filters; — — —, ^{14}C -RNA. c, Resedimentation profile of the heaviest *trp* mRNA species of the mutant SO-584 (*trpOAB673*). X, counts retained on *pt77*-DNA filters, minus counts retained on ϕ 80-DNA filters; Δ , counts retained on *ptE*-DNA filters, minus counts retained on ϕ 80-DNA filters; — — —, ^{14}C -RNA.

in good agreement with the results in Fig. 3 (column 6 in Table 2). The results obtained with the multisite mutants are in marked contrast to those obtained with the control and clearly support the idea that they carry deletions in the early portion of the *trp* operon. The relative sizes of these deletions are in good agreement with those indicated by the genetic data.

If these deletions extend past *trpP1* and join the remainder of the *trp* operon to an outside promoter they should show no recombination in crosses to the promoter mutation *trpP1443* (Fig. 1). A full description of this mutation will be published separately (Angelosanto *et al.* unpublished observations). Results of these crosses given in Table 3 show that recombination occurs in all cases and, as expected, is higher with deletion *trpAB688*, which does not extend into the operator, than with the others. Of these *trpOAS26* shows a higher recombination frequency than either of the two *trpOAB* deletions, which also make lower constitutive levels of both TSase and *trp* mRNA (Table 1). These results suggest that the *trpOAB* deletions extend partially into *trpP1* but *trpOAS26* does not (Fig. 1). A close examination of Table 1 reveals that while *trpOAS26* makes between two and three times more *trp* mRNA than either *trpOAB-673* or *trpOAB1349*, it synthesises between 20 and 30 times more TSase than the latter two deletion mutants. Since *trpOAS26* ends before *trpB* while the *trpOAB* deletions penetrate into *trpB*, the differences in TSase levels observed these strains could be an indication that translation of the *trp* operon begins at initiators of different efficiencies in these mutants.

Table 3 Recombination frequencies observed in crosses between the promoter mutation *trpP1443* and several deletions

Cross no.	Recipient strain	Genotype	Frequency of recombinants 10^{-5}
1	SO-160	<i>trpOAS26</i>	26
2	SO-441	<i>trpOAB1349</i>	3
3	SO-584	<i>trpOAB673</i>	1
4	SO-645	<i>trpAB688</i>	460

The crosses were carried out by transduction using the mutant L7 of bacteriophage P22 (ref. 24). To increase recombination frequencies in crosses 1, 2 and 3 the phage lysates were irradiated for 160 s with a GE germicidal bulb (15 W, polished reflector) at a distance of 60 cm. These were optimal conditions for recombination, since phage lysates so treated showed a five-fold increase in recombination frequency over unirradiated phage²⁵. In all crosses *trpP1443* was the donor and can grow slowly on minimal medium by forming pinpoint colonies after 48 h of incubation at 37° C. Thus recombinants carrying *trpP1443* can be recognised by their typical phenotype. The frequency of recombination is expressed as the number of prototrophs over total number of recombinants (prototroph + mutant) recovered. In all cases, control platings of uninfected bacteria as well as phage lysates were carried out.

All the results we have presented consistently indicate that, in the *trp* operon of *S. typhimurium*, the *trp* operator (*trpO*) and the *trp* promoter (*trpP1*) are essentially separable and arranged in the order *P1-0* in relation to the first structural gene *trpA*. Brammar *et al.* (personal communication) have reached the same conclusion for the *trp* operon of *E. coli*. Our data also show that deletion of most or part of *trpA* does not alter the regulation of the *trp* operon (that is *trpAB688* and *trpAB674*) but this is altered if the deletion includes *trpO*. This gives further support to earlier findings^{5,13,14} that the first structural gene of the *trp* operon does not play an essential role in the regulation of the expression of the *trp* operon.

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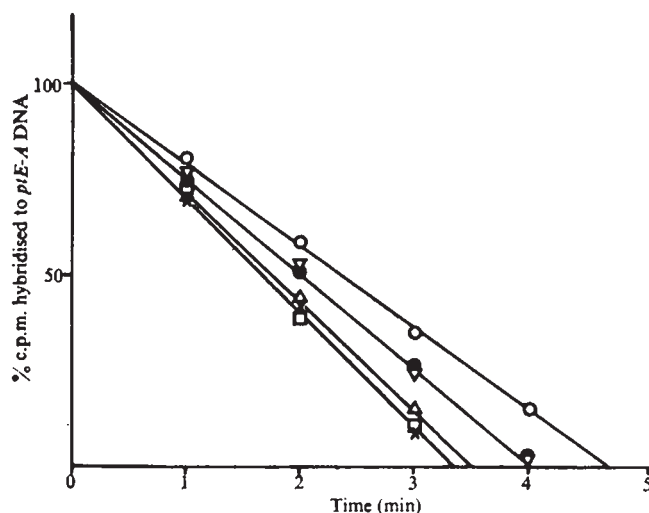


Fig. 3 Transcription of the *trp* operon after the addition of rifampicin. Samples (4 ml) of overnight L broth cultures were used to inoculate 100 ml of fresh L broth, and the cultures incubated with aeration for 2 h at 37° C. The cells were collected, washed with 0.03 M Tris-HCl buffer, pH 7.9, suspended in 25 ml of the same buffer and incubated on a shaker for 2 min at 30° C. At this point, 0.25 ml of a 0.1 M EDTA solution was added to the cell suspension, and incubation under the same conditions was continued for a further 2 minutes. Pretreatment of the cells with EDTA in Tris-HCl¹⁸ made the cells susceptible to immediate inhibition by moderate concentrations (20 $\mu\text{g ml}^{-1}$) of rifampicin¹⁹. At the end of this period, 75 ml of 4/3 strength Vogel and Bonner's minimal medium¹⁶ supplemented with 1% glucose were added and incubation continued. At the end of 10.5 min rifampicin (30 $\mu\text{g ml}^{-1}$) was added to the culture (time zero). Samples (10 ml) were removed at various times and pulse labelled for 30 s with ³H-uridine (40.4 Ci mmol⁻¹) and killed as described in the legend to Table 1. ³H-RNA was isolated and hybridised with denatured DNA of *pE-A* transducing phage. The percentage of c.p.m. of ³H-RNA hybridised is plotted at the midpoint of each pulse period after subtraction of the counts hybridised to $\phi 80$ phage DNA. ○, *trpD9*; ●, *trpOAS26*; ×, *trpOAB1439*; △, *OAB673*; ▽, *trpAB674*; ■, *trpAB688*.

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letters to nature

Observation of the 3_{12} – 3_{13} transition of interstellar formaldehyde

ALTHOUGH several transitions of formaldehyde have been observed in interstellar space^{1–7}, there has been only one prior report of an observation of the 3_{12} – 3_{13} transition³. An energy level diagram of the lower rotational levels indicating the transitions that have been detected is given in Fig. 1. The $\Delta J=1$ transitions are seen in emission. The $\Delta J=0$ transitions are seen in absorption and emission^{7–9} with a trend for the higher J transitions to be seen in emission (additional confirmation of this is provided by the unpublished work of B. Turner who has seen the 4_{13} – 4_{14} transition in emission). Here we report emission from the 3_{12} – 3_{13} transition at 28.975 GHz in Sagittarius A (Sgr A).

Observations were made with the 4.57-m Cassegrain paraboloid at the University of British Columbia. The receiver was operated in a beam-switched mode with the half-power beamwidth being 10'. The first stage of the receiver was a balanced mixer with a double sideband system temperature of 700 K. In this work, however, losses from the switch, which was not operating at its centre frequency, and ground radiation degraded the system temperature by a factor of two. Calibrations of antenna temperature were made using a gas discharge tube which in turn was calibrated using thermal sources at liquid nitrogen and ambient temperature. Spectral line data were taken with a filter spectrometer having 32 contiguous channels each of 1 MHz bandwidth.

The measured spectrum (Fig. 2) is the average of two equal amounts of data one of which had been shifted in frequency by 3 MHz. The data were obtained over seven nights of clear weather and represent 6.93 h of integration on Sgr A. Also shown for comparison is an 'off-source' spectrum representing 6.58 h of integration. The off-source data were obtained in exactly the same manner as the on-source data, except that the feed horns were covered by absorbing material because of poor weather. The much larger noise in the formaldehyde spectrum is presumably due to fluctuations in atmospheric emission at the large zenith angle ($\sim 80^\circ$) at which we observe Sgr A. The result we claim is an emission feature with a maximum antenna temperature of ~ 0.5 K centred at 0 km s^{-1} and extending about $\pm 30 \text{ km s}^{-1}$. The significance of the result is 3σ and so it should be treated with caution until confirmed by other observers. The feature at -82 km s^{-1} , although almost as significant as the broad feature centred at 0 km s^{-1} , is dependent upon a single datum point and is probably a spurious signal. The probability of this happening in a single channel is much higher than in five adjacent channels.

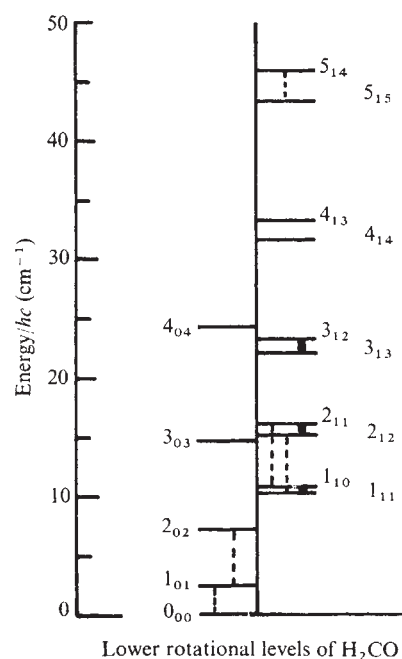


Fig. 1 Observed transitions of H_2CO are indicated in the energy level diagram. Transitions observed in emission are indicated by dashed lines while those observed in absorption are indicated by full lines.

The velocity range covered by the emission line is somewhat different from the 6 cm formaldehyde absorption profile in Sgr A (ref. 10) where the predominant absorption feature occurs over the range $+20$ to $+65 \text{ km s}^{-1}$. Other molecules such as NH_3 (ref. 11), Xogen (ref. 12), CO (ref. 13) and CH_3OH (ref. 14) also give emission at positive velocities. The 6 cm formaldehyde absorption profile, however, does have one component at 0 km s^{-1} but this is narrower than our emission result. There is also agreement in velocity with the emission and absorption spectrum of HI (refs 15, 16). These agreements in velocity suggest a physical association of the emitting clouds.

There are now nine different transitions that have been observed from $\text{H}_2^{13}\text{C}^{18}\text{O}$, but most of these have not been studied very extensively. The 3_{12} – 3_{13} line should be confirmed and more data should be obtained on the sizes and distribution of the $\Delta J=0$ emitting regions in order to have a clearer understanding of the factors involved in the excitation process.

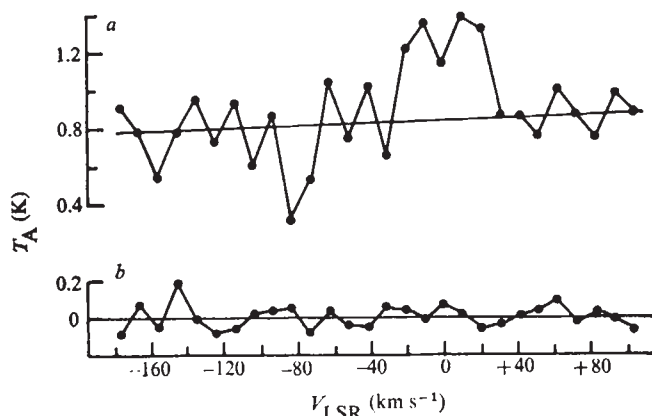


Fig. 2 Line profile of formaldehyde and control spectrum. *a*, $J_k=3_{12}-3_{13}$, time 6.93 h; *b*, off-source, time 6.58 h.

In the course of these observations we also searched for the $J_k=1_0-0_1$ transition of SO at 30.001 GHz in the molecular source in the Orion Nebula and obtained an upper limit to our antenna temperature of 0.2 K. The 4_3-3_2 and 3_2-2_1 transitions have previously been observed¹⁷, and if the column densities were the same at 30 GHz as at 99.3 GHz, we would expect an antenna temperature of 0.6–1.2 K. Our upper limit implies an upper limit to the angular size of the SO emitting region $\sim 5'$ which is comparable to the sizes of the HII region and the H₂CO 2 mm emitting region.

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Evolution of the Moon's orbit and the origin of life

PERIODICITIES in fossil corals may be used to determine the number of days in a year¹; both daily and seasonal variations are visible in the growth patterns. Scrutton² used

similar data to determine the number of days in a lunar month; monthly and fortnightly periodicities are present because of tides. The available data^{3–6} through the Palaeozoic are given in Fig. 1*a* and *b*. We conclude, from studies of living crustaceans and recent fossils, that the growth patterns are sensitive to the solar day. So, the data represent the number of solar days in a year and in a lunar month; the present values are 365.25 and 29.53 d respectively. Panella³ has also used periodicities in stromatolites to determine the number of solar days in a lunar month during the Precambrian. His results are given in Fig. 1*c*.

Palaeontological data can be used to determine the past history of the lunar orbit^{7–9}. Assuming conservation of angular momentum in the Earth–Moon system the length

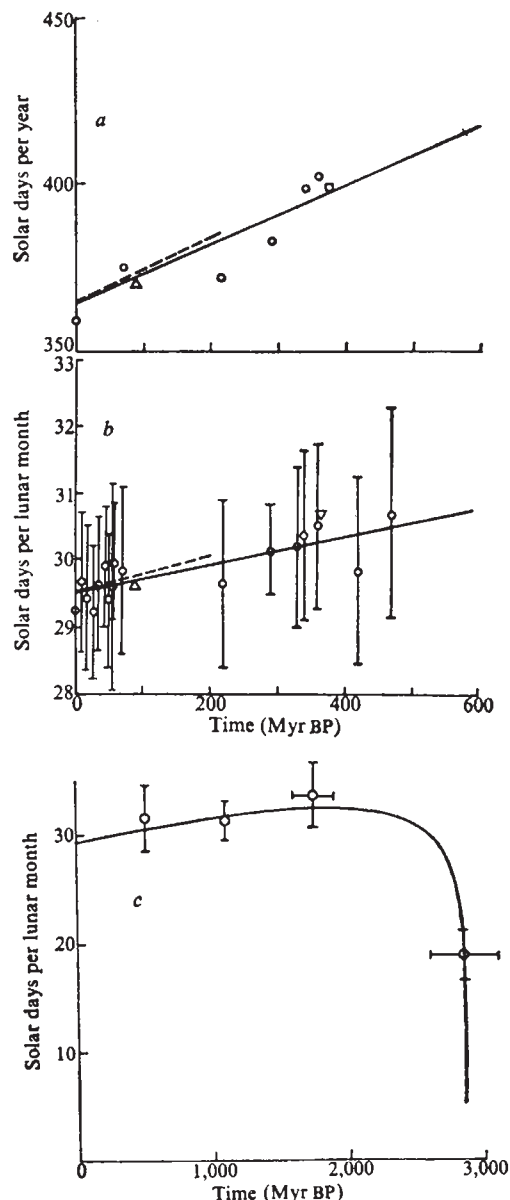


Fig. 1 *a*, Data on the number of solar days in a year from molluscan shells is compared with the dependence we predicted and the value deduced from satellite observations (Newton¹¹). *b*, Data on the number of solar days in a lunar month as in *a*. Error bars are standard deviations of the data. *c*, Data on the number of solar days in a lunar month from stromatolites (Pannella³) compared with the predicted dependence from our work. The error bars on the counts are the standard deviations of the data; the error bars on the time are the stated errors on the dating of the fossils. $\circ$, Ref. 3; $\square$, ref. 4; $\triangle$ 5; ref. 6; — — —, ref. 11; —, our results.

of the solar day and lunar month can be related; it is also assumed that the length of the year remains constant. A lunar month cannot have more than 32.5 solar days (refs 8, 10). Because the Precambrian stromatolite data given by Pannella³ yield only monthly data, he could not show that the number of days in a month was not a multiple of the values given in Fig. 1c. But, the dynamical constraint on the number of days in a month places an upper limit which precludes, within the standard deviation of his data, an alternative interpretation of the data.

With an assumption on the rate of tidal dissipation, the time evolution of the lunar orbit and length of day can be determined. If the dissipation is proportional to the tidal force the rate of energy dissipation is proportional to the sixth power of the Earth-Moon separation. Varying the single unknown parameter, the constant of proportionality between the tidal force and the tidal dissipation, the best fit to the data is given by the solid lines in Fig. 1a, b and c. In determining these curves the Bulawayan data at 2,850 Myr BP are critically important. Without these data a close approach could not be predicted. The counts of the Bulawayan growth patterns favour the 18.7 d month since there is a strong peak at 9 d corresponding to the fortnightly tides. The alternative interpretation of a 37.4 d month falls outside the allowed maximum number of solar days in a lunar month even when the full standard deviation of the data is subtracted.

The predicted value for the present tidal dissipation is 2.58×10^{19} erg s⁻¹. The corresponding value for the present acceleration of the moon is $\dot{n} = -19.3$ and the value for the present acceleration of the Earth's rotation

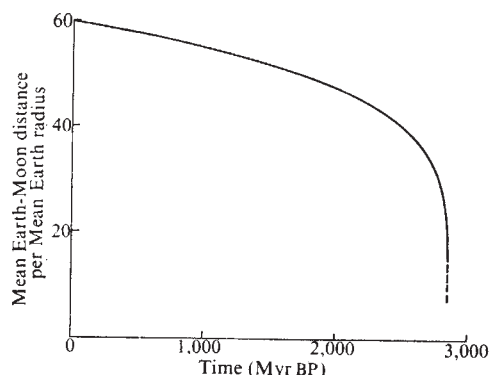


Fig. 2 Predicted dependence of the Earth-Moon separation on time.

is $\dot{\omega} = -934$ s (100 yr)⁻². These values are in excellent agreement with the value for the acceleration of the Moon obtained from satellite observations¹¹. Newton's preferred value for the present lunar acceleration is $\dot{n} = -22.4$ s (100 yr)⁻² and the corresponding changes in the number of solar days in a year and in a lunar month are given in Fig. 1a and b. Jeffreys¹² has reviewed the measurements of lunar acceleration and acceleration of the Earth's rotation and favours the value given by Newton. The assumption that the dependence of the tidal dissipation on the tidal force is independent of time on the time scale of 10^9 yr certainly cannot be justified in detail. But the resulting calculations are consistent with observation. In Fig. 2 the dependence of the Earth-Moon separation on time is given. It seems that the Moon approached the Earth $2,850 \pm 250$ Myr BP. If the calculations of Gerstenkorn¹³ are accepted this close approach was near the Roche limit for tidal catastrophe. In any case, a near approach would result in very large tides and large energy dissipation. Tidal heating could possibly have vaporised the oceans. This has both geological and biological implications.

There is evidence in the geological record¹⁴ that a pulse of high temperature volcanism occurred about 2,800 Myr BP. Many of the high temperature intrusions of ultrabasic rocks are dated at this time. Such intrusions could be the result of tidal heating near the surface.

The implied catastrophe roughly coincides with the first records of life. The Onverwacht Series of southern Africa, which contains the first cell-like objects¹⁵⁻¹⁸ is, contrary to earlier estimates¹⁹, at least 3,000 Myr old (ref. 20) and at most 3,200 to 3,300 Myr old (refs 21, 22). The oldest extensive Banded Iron Formation, one indirect indication of life²³, has been reliably dated²⁴ at no more than 3,000 Myr BP. The earliest conclusive evidence for life is, in fact, the Bulawayan stromatolites²⁵⁻²⁷. Though stromatolites generally form in warm waters, it has been suggested that many Precambrian-type stromatolites formed in scalding waters^{28,29}, which is consistent with the idea of thermal catastrophe. It seems within the realm of possibility that a global thermal event might have been involved in the origin of life. On a grand scale, one such event would resemble experiments performed by Fox^{30,31} in which heating and cooling of 'organic soup' leads to the formation of remarkably cell-like coacervates.

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Relative abundances of positrons and antiprotons in the primary cosmic ray flux

SHEN and Berkey¹ calculated the ratio of antiprotons and positrons to be expected in the primary cosmic rays assuming that both components are the end products of proton-proton interactions, and pointed out that the observations of the positron spectrum would permit us to deduce the antiproton energy spectrum. Observations on the ratio $e^+/(e^+ + e^-)$ have been made² in the 5–50 GeV range, and we have used the results to put an upper limit on $\bar{p}/p$ at various energies.

Our calculations on the expected antiprotons-positron ratio differ in one significant respect from those of Shen and Berkey¹: we have detailed measurements of the cross section of antiproton production up to intersecting storage ring energies. That permitted us to calculate the $e^+/\bar{p}$ ratio quite reliably.

We have plotted the latest $\bar{p}$ and π^+ production cross-sections (Fig. 1). They were deduced from the work of Antinucci *et al.*³ and Ramaty and Lingenfelter⁴.

It has been shown^{5,6} that $\langle E_p \rangle / E_p$, the ratio of the mean energy of antiprotons, $\langle E_p \rangle$, to incident-proton energy, E_p , is approximately 9, independent of the energy of the incident protons.

This differs significantly from the results of Shen and Berkey¹ who found this ratio to vary as $E_p^{1/4}$. For the purpose of this calculation, we can summarise the results of the kinematics:

$$\left. \begin{aligned} \sigma_{\bar{p}}(E_p) &\simeq 9.3 \times 10^{-3} (E_p - 10.8) \text{ mb} \\ \langle E_p \rangle &\simeq 9 E_p \text{ GeV} \end{aligned} \right\} 20 \lesssim E_p \lesssim 550 \text{ GeV} \quad (1)$$

$$\left. \begin{aligned} \sigma_{\pi^+}(E_p) &= 27.7 E_p^{1/4} \text{ mb} \\ \langle E_{e^+} \rangle &= 0.175 E_p^{3/4} \text{ GeV} \end{aligned} \right\} 50 \lesssim E_p \lesssim 1,500 \text{ GeV} \quad (2)$$

Following a procedure analogous to that of Shen and Berkey¹ we found that the ratio of the production spectrum of positrons to antiprotons can be written as:

$$\frac{Q_{e^+}(E)}{Q_{\bar{p}}(E)} = \frac{6.176 \times 10^{-2} E^{-0.586}}{2} \times \frac{\sigma_{\pi^+}[(22.86 E^{4/3})]}{\sigma_{\bar{p}}(9E)} \quad (3)$$

which gives rise to values for the ratio $e^+/\bar{p}$ of 4 at 5 GeV and 1.56 at 10 GeV. These values depend on the spectral index of the differential proton energy spectrum only, taken to be $1.9 \times 10^4 E^{-2.75} \text{ m}^{-2} \text{ sr}^{-1} \text{ s}^{-1} \text{ GeV}^{-1}$ (refs 7 and 8). The factor of two in the denominator comes from the decay of antineutrons in space.

The equilibrium value of $e^+/\bar{p}$ will depend on the actual model of propagation and would tend to lower somewhat the ratio $e^+/\bar{p}$. Similarly, if the leakage mean free path is energy

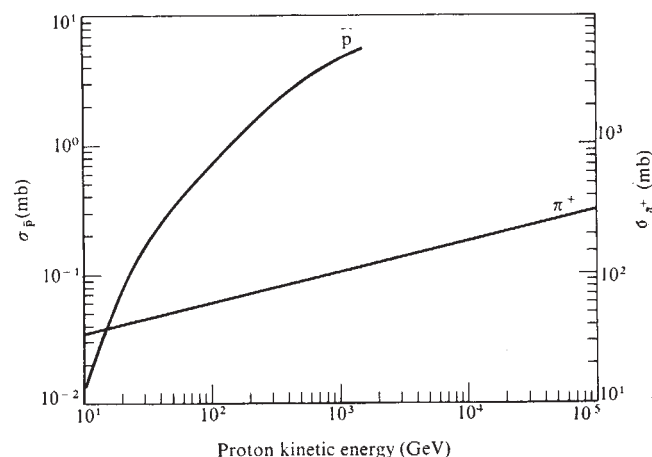


Fig. 1 The production cross sections of π^+ (scaled down by a factor of 1.5) and $\bar{p}$, as a function of proton kinetic energy in proton-proton collisions.

dependent this ratio will again be lowered. We will ignore these effects for the present and assume that the production and equilibrium values are the same. They will change the ratio by a factor of two or less.

We interpret the results of Buffington *et al.*² as indicating an upper limit of 10% on the ratio $e^+/(e^+ + e^-)$ in order to obtain an upper limit on p/p . That gives an upper limit on the e^+ energy spectrum when combined with the measurements of $(e^+ + e^-)$ as summarised by Müller and Meyer⁹. Thus,

$$\begin{aligned} p/p &\lesssim 1.8 \times 10^{-4} \text{ at 5 GeV} \\ &\lesssim 5.7 \times 10^{-4} \text{ at 10 GeV} \end{aligned}$$

which, even after allowing for propagation uncertainties, is about an order of magnitude lower than the value of 6×10^{-3} obtained by Bogomolov *et al.*¹⁰ in a larger GeV range.

As the e^+/p ratio produced in nuclear reactions is very well known, measurements of e^+/p (in conjunction with knowledge of the proton flux itself) depend only on the amount of e^+ from cosmic ray sources and on the energy dependence of the storage time of primary protons in the galaxy. Comparison of e^+ and p fluxes would be extremely valuable in the understanding of cosmic ray production and propagation.

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Will there be a large earthquake in central California during the next two decades?

P-VELOCITY decreases before earthquakes¹⁻⁴ can be observed by monitoring the average P-residual at seismic stations near the focus⁵⁻⁷. The P-residual is the difference between the observed travel time from an epicentre to a station and that computed theoretically on the basis of some Earth model and a least squares estimate of the earthquake location and origin time. If the P-velocity in the crust underneath a seismic station is decreased temporarily, each seismic wave will arrive somewhat later during the pre-monitory period. Before three thrust^{5,6,18} and one strike-slip event⁷ the delay was found to be approximately 0.4 s.

Here I compare the average teleseismic P-residuals of four stations located near the San Andreas fault (Berkeley, BKS; Mt Hamilton, MHC; Priest, PRI; and Pasadena, PAS) to those of three stations to the East of it (Mineral, MIN; Jamestown, JAS, and; Golden, GOL) (Fig. 1). Since our interest is focused on the detection of precursor anomalies lasting several years (earthquake magnitudes ≥ 6.5) Fig. 2 shows annual mean residuals. The standard deviations of the annual means are approximately 0.1 s for 1961–63, and 0.07 s to 0.04 s since then, shown by error bars in the figures.

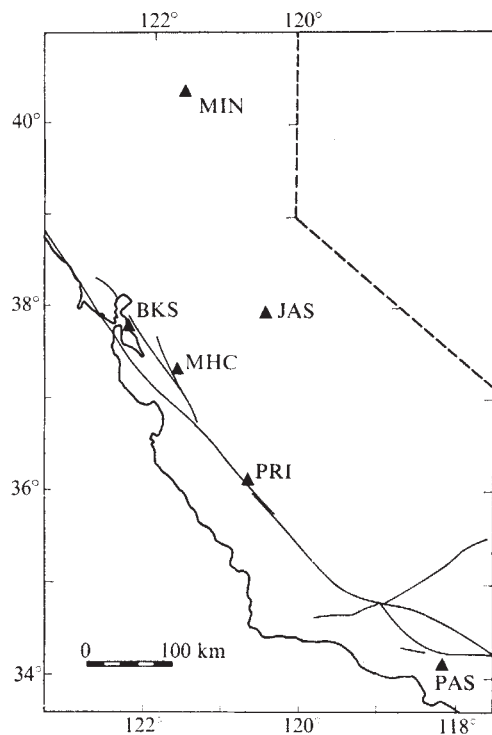


Fig. 1 Map of California showing the locations of seismic stations with respect to the San Andreas fault. The station Golden is located in Colorado at $39^{\circ} 42'N$ and $105^{\circ} 22'W$.

The constancy of the average P-residuals in Fig. 2 is surprising compared with other data⁵⁻⁷ where fluctuations of 0.15 s are quite common and where precursor anomalies have amplitudes of 0.4 s. Only stations PRI and PAS show disturbances of more than 0.2 s from the long term

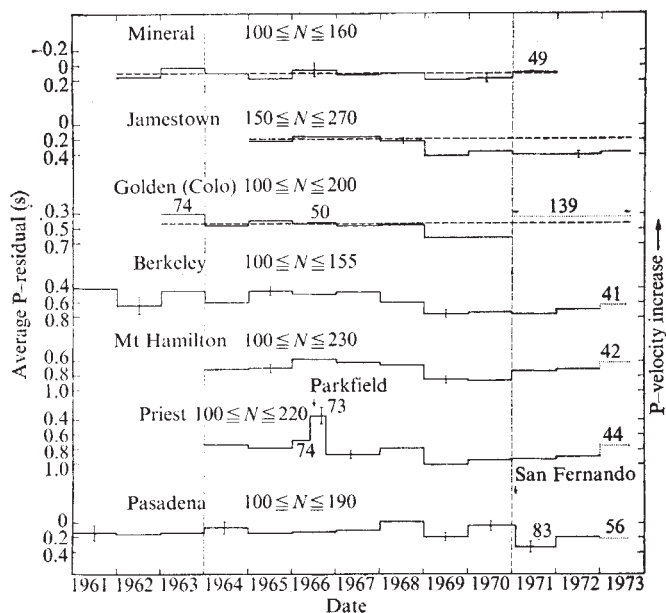


Fig. 2 Mean annual teleseismic P-residuals from 3 reference (top) and 4 tectonic (bottom) stations. Dashed lines, long term average through 1968. Arrows indicate time of occurrence of local earthquakes. Vertical dashed lines separate different data sets. The data for the years 1961-1963 are from ref. 8; those for 1964-70, from ref. 9; and those for 1971-73, from ref. 10. All residuals used were from earthquakes with depths ≥ 100 km, located at distances $\geq 15^{\circ}$ whose hypocentres were determined on the basis of more than 25 readings and with absolute values smaller than 3 s.

mean. Both disturbances accompanied earthquakes near those stations. All curves except that for MIN show an increase of approximately 0.2 s in the mean annual P-residual in 1969. Clearly the increase in 1969 was due to changes in the earthquake locating procedure, since a similar change occurs at stations in other parts of the world. Therefore I assume that the stations GOL, JAS and MIN are in a different tectonic region than the San Andreas stations and thus these three observatories can be used as control stations.

Errors related to the seismic source, the teleseismic ray path, or the data procurement procedure are reduced by taking the difference between the average residuals at different stations (for details see ref. 6). Each tectonic station was compared simultaneously to as many of the three control stations as were operating each year. For this purpose a correction factor was computed which was the average of the control station deviations from their respective long term means. The average residual minus the correction factor is shown in Fig. 3.

The stations BKS, MHC, PRI and JAS are operated on a common time base by the University of California, Berkeley, and the same observer reads the arrival times at all stations. For these stations the comparison with the control station JAS will therefore be especially effective in reducing all errors except the effects of changes in the local crust and the unlikely possibility of changes in the instrument characteristics. The corrected P-residuals shown in Fig. 3 are very stable with deviations from the respective long term means of less than 0.13 s for all years, except at the times of the Parkfield and San Fernando earthquakes. (The averages for 1973 do not contain enough data points to be considered reliable.)

The decrease of the average residual at PAS before the

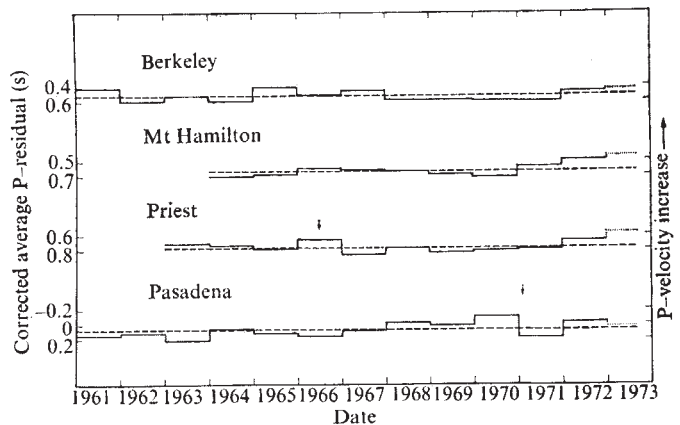


Fig. 3 Corrected annual mean residuals for 4 tectonic stations.

San Fernando earthquake suggests that PAS was located just outside the dilatant region which preceded this earthquake³, and that the crustal P-velocity under PAS increased temporarily. This may have been a consequence of stresses generated by the dilatancy closing rock pores at the periphery. After the earthquake a somewhat lower than average velocity is indicated by the P-residuals. This may have been due to partial dilatancy outside the source region caused by the redistribution of stress associated with the earthquake. Such an effect is expected from laboratory experiments¹³ and was observed for the 1966 Gisborne earthquake in New Zealand⁶. The results presented for PAS are in agreement with the constancy of the P-velocity as determined from records of quarry blasts¹⁸ and individual teleseisms¹⁷.

From Fig. 3 it is clear that, except for the minor perturbations discussed above, there is no significant change

in P-velocity in a vertical direction beneath any of these stations. McEvilly and Johnson¹⁴ concluded from explosion studies that there was no variation in both the P and S-velocities in a horizontal direction in central California. In addition they found v_p/v_s to be normal.

The absence of a P-residual anomaly in central California can be interpreted to mean that strike-slip tectonism does not produce significant precursory changes in the seismic velocity. But it seems that the P-residuals increased before the strike-slip earthquakes at Sitka, 1972 (ref. 7), and at Bear Valley, 1972 (ref. 15). These two observations suggest that the above interpretation of the data may not be correct. Since magnitude 7 and 8 earthquakes are expected to be preceded by anomalies lasting 7 and 25 years respectively^{3,12}, the data presented here and in ref. 14 suggest that no earthquake of $M > 7$ and no earthquake of $M \approx 8$ will occur between San Francisco and Parkfield, California during the next 7 and 25 yr, respectively.

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Is Kakangari a unique chondrite?

THE Kakangari meteorite, possibly a unique member of a distinct chemical class of chondrite, fell in India in 1890. Two stones were seen to fall, and though one was soon destroyed the bulk of the other, about 310 g, is preserved in Calcutta and London^{1,2}. Mason and Wiik³ concluded that Kakangari is probably a carbonaceous chondrite. Subsequent improved understanding of chemical fractionation between chondritic groups (see ref. 4) enables us to show, however, that their analysis in fact indicates that Kakangari is similar in bulk composition to the ordinary chondrites, apart from its high S content (Table 1). In this last respect it resembles carbonaceous or enstatite chondrites. The $\text{FeO} \times 100/(\text{FeO} + \text{MgO})$ molecular ratio, 13.7, lies between that of H group (bronzite)

Table 1 Composition of chondrites*.

	C†	O	K	E
Mg	10,500	9,500	9,900	7,500
Ca	730	480	489	355
Al	850	610	726	480
S	5,000-1,200	1,000	2,800	3,000-1,500‡

*Atoms, per 10^4 Si atom.

†C, carbonaceous chondrites; O, ordinary chondrites; K, Kakangari; E, enstatite chondrites.

‡The high figures are for C1 and E3, 4, respectively. Kakangari data from ref. 3; the remainder from ref. 12.

chondrites and enstatite chondrites. That is confirmed by our microprobe analyses discussed here. The mineralogical data are based on preliminary microprobe analyses on a thin section cut from a British Museum (Natural History) specimen (BM 69062).

Olivine is apparently less abundant than pyroxene. Analyses of 26 grains from 15 locations show the compositional range to be $\text{Fa}_{3-9.5}$; the grains have less than 0.1% CaO (weight) and in this respect are similar to the olivines of ordinary chondrites⁵ and distinct from those of carbonaceous chondrites⁶. Mason³ used a diffractometric method to determine the composition of Kakangari olivine and from the skewed peak he obtained, suggested that the olivine is dominantly forsteritic with some scatter towards more iron-rich compositions, perhaps Fa_{25} . Our microprobe work does not substantiate this and an X-ray powder photograph shows that the groundmass is dominantly enstatite with some troilite. That eliminates the groundmass as a possible source of olivine, as in type III carbonaceous chondrites. In any case, the compositional distribution of Kakangari olivines is more limited than in type III carbonaceous chondrites⁷; it lacks a peak at Fa_1 or the more Fe-rich grains to Fa_{50} or more. The mean olivine composition in Kakangari— $\text{Fa}_{4.9}$ —is, however, close to that of the carbonaceous chondrite Vigarano, $\text{Fa}_{5.6}$ (ref. 7).

Fifteen analyses of pyroxene from 11 locations indicate that the compositional range is $\text{Fs}_{4.6-14.5}$, with the wollastonite component from $\text{Wo}_{0.2-6.6}$; grains from 7 of the 11 locations (a location is a chondrule or grain in matrix) were in the range $\text{Fs}_{4.5-5.5}$. The mean pyroxene composition is $\text{Fs}_{6.9}$ and is similar to that of carbonaceous chondrites (ref. 7).

Troilite is ubiquitous, occurring as grains and veins in chondrules, and as grains or fine grained material in the groundmass. Its Ni content is less than 0.2% by weight, but grains in the groundmass have $0.19 \pm 0.05\%$ Cr (weight) and a grain in one chondrule has $0.43 \pm 0.05\%$. The Ti content is less than 0.05%. Kamacite occurs within chondrules, as rims ('armour') to chondrules, and as grains in the matrix. Ni and Co contents are close to 6% and 0.7% (weight), respectively. In our cursory examination, taenite was not found in the matrix, but it commonly occurs as grains in chondrules. Most grains have a fairly uniform Ni content in the range 29-32%, but one zoned grain has a core with 26% increasing to 31% or more at the rim, indicating some attempt at equilibration during falling temperature⁸. Taenite has a Co content of about 0.31%, and will be studied in greater detail.

This meteorite has a state of reduction between that of the H group (bronzite) chondrites and enstatite chondrites. In ordinary chondrites, Cr is lithophile, the sulphide containing not more than a few hundred p.p.m. of this element (ref. 9, and C. J. Elliott, personal communication) but in the enstatite chondrites, the troilite has about 1% Cr by weight and the remainder of this element occurs as the sulphide, daubréelite¹⁰. In enstatite chondrites, Ti, is also largely chalcophile, the bulk, about 75%, occurring as a minor constituent of troilite¹¹. The presence of significant Cr and absence of Ti in the Kakangari troilite thus indicate a state of reduction between that of enstatite and ordinary chondrites. This conclusion is also

supported by the oxidised Fe content of the olivine and pyroxene; the enstatite chondrites have almost no Fe in the silicate¹⁰ whereas ordinary chondrites have olivines and pyroxenes in the range Fa_{16-33} and Fs_{14-25} , respectively (6). The mineral chemistry thus supports our interpretation of Mason and Wiik's³ bulk chemical analysis as indicating that Kakangari represents a class of chondrite previously unrecognised.

Because it is apparently unique, the limits (if any) of the chemical properties diagnostic of its class cannot yet be determined. Although closer to the L group of ordinary chondrites with regard to its low (22.47%) total Fe content, its oxidation state indicates a closer relationship with the H group. It is chemically distinct from enstatite and carbonaceous groups. Perhaps it is best to consider this stone as representative of a K group of chondrites, the diagnostic properties of which are undefined.

A. W. R. and J. C. Bevan, and R. F. Symes, are thanked for sample preparation or help with microprobe analyses. E. E. Fejer arranged the X-ray powder photograph.

Note added in proof: We now realise that Kakangari may be chemically related to the recrystallised chondrite Mount Morris (Wisconsin), to the silicate portion of the stony-iron Winona, and to chondritic xenoliths in the achondrite Cumberland Falls; we have begun to investigate this.

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A Cretaceous guyot in the Rockall Trough

THE Anton Dohrn seamount^{1,2} is situated in the central part of the Rockall Trough (Fig. 1). The feature is of some interest in view of its relatively isolated position in the trough and because it has an almost flat summit, a rare characteristic for a seamount in the North Atlantic. Because it has received little attention since its early bathymetric survey, we took the opportunity of recording magnetic and seismic profiles over it and also carried out a preliminary sampling investigation in the area during a recent cruise of *RRS John Murray*. In this contribution we report some of our findings and discuss their significance in relation to the history of the region.

The seamount possesses a strong magnetic signature (Fig. 2) in marked contrast to the main part of the Rockall Trough which is magnetically quiet^{3,6}. The high amplitude, short wavelength anomalies, together with the general shape of the seamount, are consistent with its being a volcanic feature. It is clearly different in composition and origin from the nonmagnetic Galicia Bank⁷, another flat-topped seamount which is situated close to the European

continental margin near 43°N. Seismic profile A₁–B₁ in Fig. 3 reveals the presence of a capping of sediments which, assuming an average sedimentary sound velocity of 2.0 km s⁻¹, reaches a thickness of over 100 m. Although the seismic evidence is not unequivocal, the contact between the sediments and the main volcanic pile appears to be defined by the reflector labelled X, the major break in slope on the seamount occurring where this horizon outcrops. A 200 kg haul of rocks obtained by dredging the steep slope just below the bench at which reflector X is exposed (site 4, Fig. 1) supports this interpretation. Over 60% by weight of the material recovered consists of ahermatypic corals (mainly *Lophelia prolifera*) and not unexpectedly, exotic blocks of glacial origin. The remaining fraction is made up of basic lavas and tuffs, many being highly altered and very friable, which are considered to have come from outcrops situated along or close to the path taken by the dredge. The composition of the central portion of the largest block of lava, one weighing 38 kg, is given in Table 1. This is a basalt of alkaline affinities which is predominantly made up of plagioclase feldspar (An₆₈–An₇₂), together with augite and hornblende, the latter replacing the pyroxene. Unfortunately, the extent of the alteration makes it unsuitable for radiometric dating.

Even though the basic volcanics are far from fresh they have proved to be of considerable interest, because chalk was found adhering to several specimens. The chalk either occupies vesicles, as in the case of the 38 kg block, or it occurs as a matrix between highly altered, angular and sometimes vesicular clasts of basalt (see Fig. 4). Despite its intimate association with the lava, the sediment shows no evidence of baking. The chalk contains abundant nanofossils whose preservation is, unfortunately, somewhat marred by calcite overgrowths. Nanofossil assemblages are assigned to the *Nephrolithus frequens* Zone of Cepek and Hay⁸ which Manivit⁹ considers to be Upper Maestrichtian in age, correlating it with the *Globotruncana contusa* foraminiferal zone. The following species were recorded: *Nephrolithus frequens*, *Lithraphidites grosspectinatus*, *L. carniolensis*, *L. quadratus*, *Tetralithus murus*, *T. quadratus*, *Markalius astroporus*, *Lucianorhabdus cayeuxi*, *Ceratolithoides kamptneri*, *Cylindralithus serratus*, *Rhabdolithina splendens*, *Reinhardtites anthophorus*, *Microrhabdulus stradneri*, *Cretarhabdus decorus*. The scarcity of members of *Lithraphidites quadratus* and *Nephrolithus frequens* together with the absence of pentoliths suggests an open ocean assemblage^{10,11}.

The time of formation of the Anton Dohrn seamount cannot be determined with any certainty without more extensive sampling but the discovery of Maestrichtian chalk so closely associated with the volcanics points to an Upper Cretaceous age. The chalk may indeed be the earliest sediment to be deposited on the seamount. Evidently, the feature was not created at the time of the main separation of the Rockall Plateau from Europe because it is characterised by large magnetic anomalies whereas most of the Rockall Trough is magnetically quiet. The Cretaceous age and the spatial relationship of the Anton Dohrn seamount with respect to the volcanic centres in north-east Ireland and Scotland make it difficult to envisage the feature as part of the scar trace of the mantle plume postulated by Duncan and others¹² to explain age variations in the Thulean province. It is, perhaps, more likely to be related to the lithospheric heating and general tensional conditions in the crust which immediately preceded the development of new ocean basins to the west and north of the Rockall microcontinent.

An appreciable proportion of the seamount was removed by erosion following the main volcanic activity, the even surface of reflector X probably being the level to which erosion extended. The central knolls (Fig. 1) are possibly remnants of resistant feeder channels within the volcano.

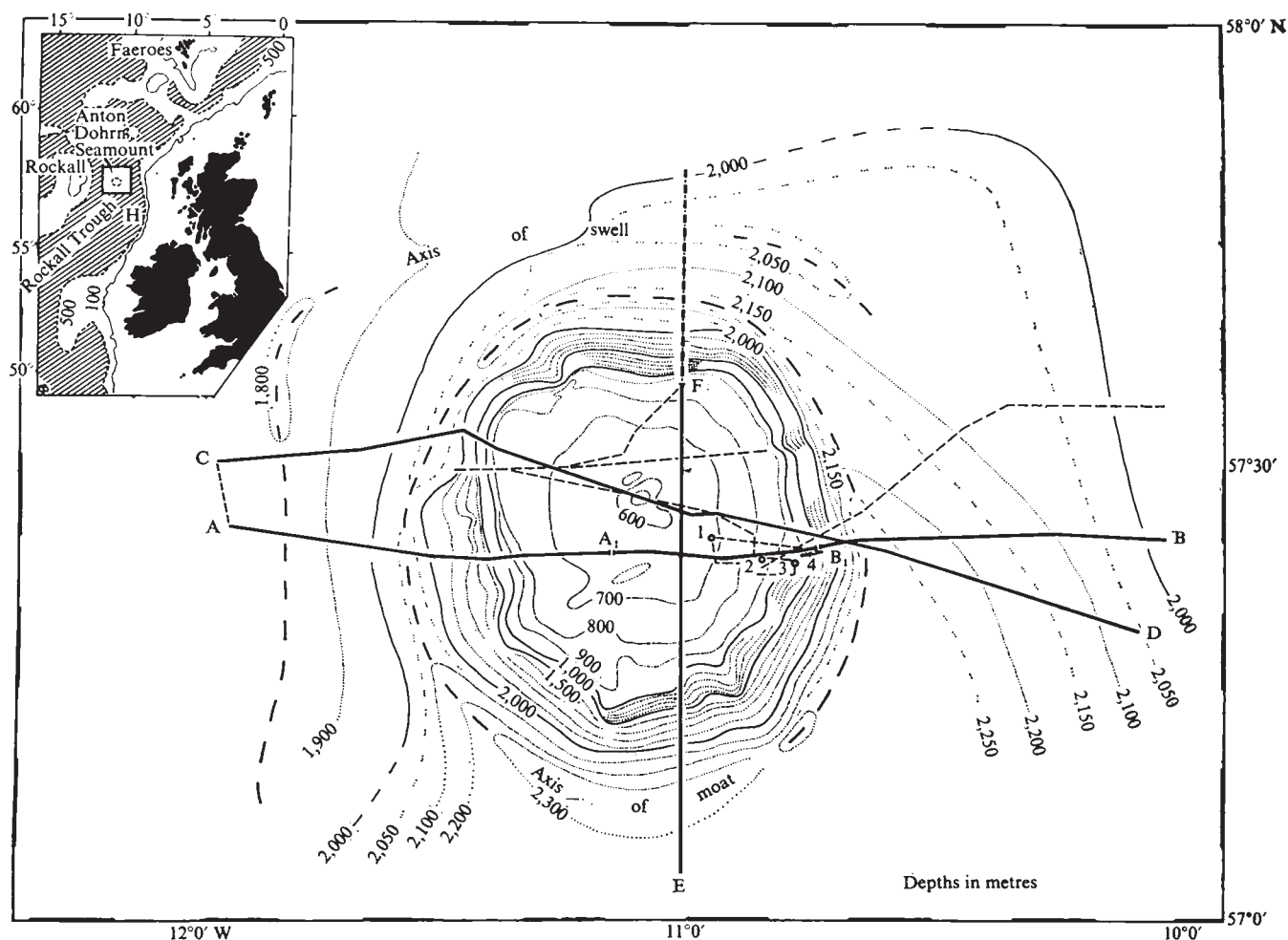


Fig. 1 Tracks of *RRS John Murray* shown on a bathymetric map of the Anton Dohrn seamount, taken from Dietrich and Ulrich⁴. Water depths are corrected for sound velocity variations in sea water using the tables of Matthews³. Profiles along portions of the track indicated by heavy lines are reproduced in Figs 2 and 3. Positions of core stations 1-3 and dredge site 4 on the eastern side of the seamount are indicated. The moat and swell around the seamount arise from non-uniform deposition of sediments controlled by the pattern of bottom currents⁴. H=Hebrides Terrace.

Similar features have also been recognised on some of the Pacific guyots¹³. A comparison of the shapes of volcanic islands and the Anton Dohrn seamount suggests that it originally stood at least 2 km above sea level. After the period of planation the volcano sank over 600 m. Its present, southeasterly tilt (Figs 2 and 3) may have been acquired at this time or it may reflect subsequent loading of the crust by thick sediments which airgun profiles indicate lie beneath the lower part of the continental slope between the seamount and the Hebrides Terrace (H in Fig. 1).

Coring was carried out upslope from the dredge site (stations 1-3; Fig. 1) to determine the time and rate of sinking of the seamount from the stratigraphy of the sediment layer above X. According to the seismic profiles, reflectors normally lying within the layer are exposed there. Unfortunately, the corer failed to penetrate a cover of stiff Glacial or post-Glacial shelly and silty clays so, at present, we can only assign a tentative date for the subsidence from what is known about vertical movements in the surrounding areas. In this region of the North Atlantic there is evidence for at least one major phase of regional

subsidence in post-Cretaceous time. Seismic profiles from the continental margin west of Britain reveal a widespread unconformity, believed to be of early Tertiary age from a limited amount of stratigraphic data, which appears to have resulted from erosion followed by downwarping of the margin and outbuilding of the continental slope^{14,15}. To the west of the Rockall Trough the sedimentary record of the Rockall Plateau provides convincing evidence of early Tertiary subsidence. The lithological variations at DSDP site 117 show that at least part of the plateau sank 600-700 m in late Palaeocene-early Eocene times at an average rate of 14 cm per 1,000 yr (ref. 16). Although the movements to the east and west of the Rockall Trough may not have been synchronous, there is general agreement that an important episode of subsidence took place between the late Cretaceous and Oligocene, possibly related to cooling of the lithosphere in this region following the birth of the Reykjanes Ridge and the early Tertiary igneous activity in the Rockall and Scottish areas. It seems probable that sinking of the Anton Dohrn seamount is yet another manifestation of these early Tertiary crustal adjust-

Table 1 Analysis of basalt dredged at site 4

	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	FeO	MgO	CaO	Na ₂ O	K ₂ O	H ₂ O	TiO ₂	P ₂ O ₅	MnO	CO ₂	Total
%	39.80	15.13	10.09	2.08	5.34	13.64	2.21	0.60	4.76	1.53	0.46	0.18	3.94	99.76

ments and is also an indicator of appreciable subsidence of much of the Rockall Trough at this time.

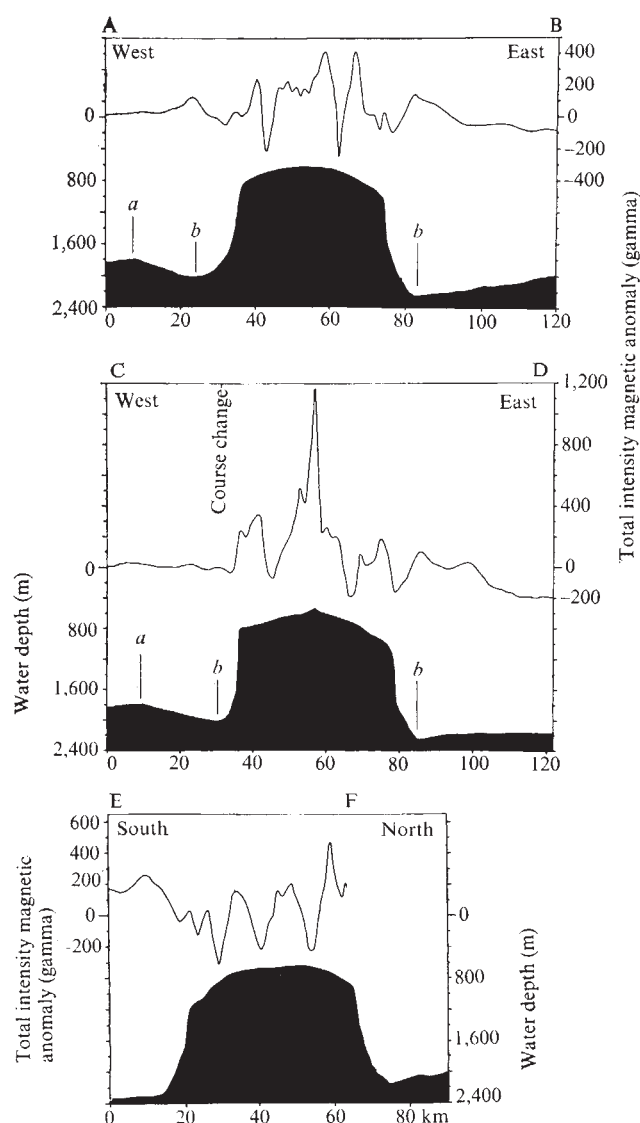


Fig. 2 Bathymetric and magnetic profiles across the Anton Dohrn seamount. The International Geomagnetic Reference Field has been subtracted from the total field observations to obtain the residual magnetic anomalies (1 nT = 1 gamma). a, Axis of swell; n, axis of moat.

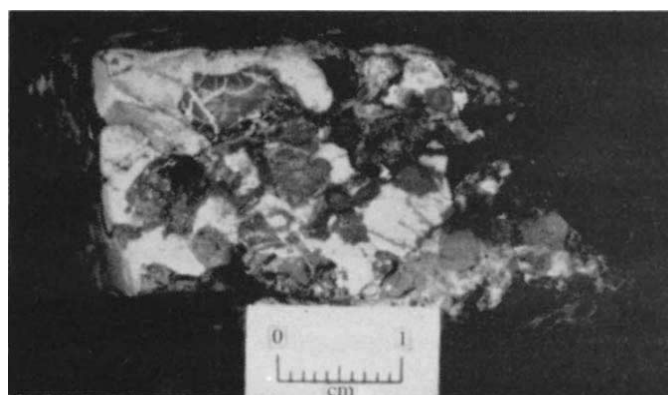


Fig. 4 A section showing the intimate association of the light-coloured Maestrichtian chalk and weathered volcanics.

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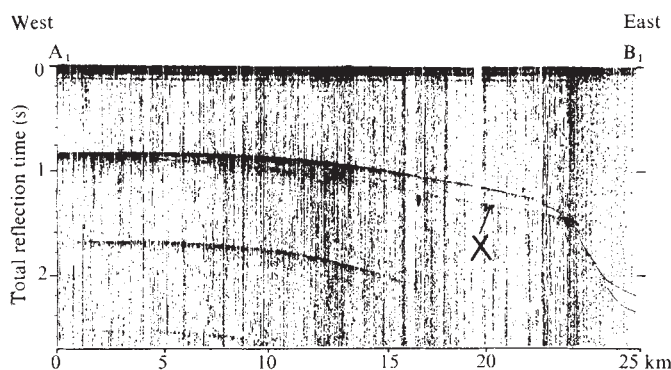


Fig. 3 Seismic reflection profile along line A₁-B₁ recorded using an airgun sound source.

A proposed γ camera

ISOTOPES which emit γ rays are used for clinical diagnosis. This has led to the development of image forming systems which are based on detectors placed in the proximity of a radiating source, allowing abnormalities such as tumours and gross lesions to be detected.

As γ rays cannot be focused, the formation of an image depends upon an estimation of particle trajectories. Generally, this necessitates the elimination of all trajectories outside a narrow range of incidence angles. This has been achieved using lead collimators. Such systems give directional information but do not indicate the distance from the detector to the source point. Clearly only two-dimensional images can be obtained. Using a collimated scintillator and photomultiplier system severely limits the use of the cameras. We here outline a completely different approach to the principles of γ -ray imaging, which seems to offer considerable advantages over existing systems.

Scanners or cameras can be used. The scanner has a detector head consisting of a scintillation crystal, a photomultiplier and a lead diverging hole collimator. The complete image forming process can take up to 30 min. In camera systems the collimated detector must cover the entire region of investigation. Mechanical scanning is not used and the scintillation positions are detected using an array of photomultipliers. This principle is adopted in the widely used Anger system¹. The interactions used in image formation are single absorption processes which result from the photoelectric effect.

In both scanner and camera imaging, speed can be increased with greater isotope activity. With a lead collimator only a small fraction of the incident radiation is useful. Also, with low energy isotopes (< 500 keV), there is considerable scattering within the examined region. There are thus inherent limitations in resolution and sensitivity.

The Compton effect is associated with multiple interactions. The position and intensity of these interactions define, within statistical limits, the trajectory of the incident photon. In principle this would allow projection back on to an image plane (Fig. 1). If the coordinates, $\mathbf{x}_1$ and $\mathbf{x}_2$, of the first two consecutive interactions are measured, and if the scattering angle, α , is known then the emission point must lie on the surface of a cone defined by all lines at angle α to the vector $\mathbf{x}_1 - \mathbf{x}_2$ through $\mathbf{x}_1$. The intersection of this cone with an image plane, generates an ellipse with parameters which are

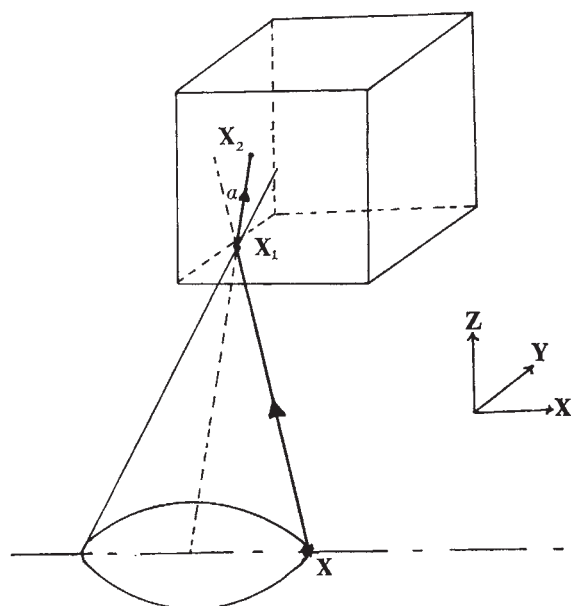


Fig. 1 The Compton γ camera principle

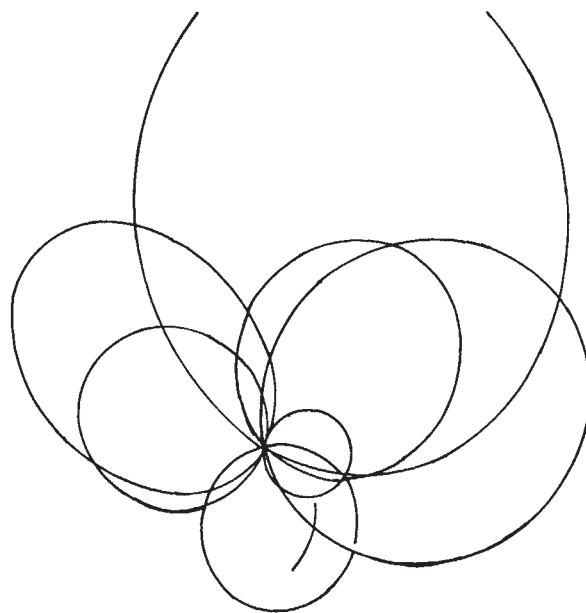


Fig. 2 Ellipses generated from a point source.

functions of $\mathbf{x}_1$, $\mathbf{x}_2$, and α . $\mathbf{x}_1$ and $\mathbf{x}_2$ can be measured directly using an array of detectors, which also give an indirect estimate of α from the energy loss, δE , at the first interaction:

$$\cos \alpha = (E - K\delta E) / (E - \delta E)$$

where E = initial source energy, and $K = 1 + (mc^2 / E)$, constant for a particular source.

If a single point source of activity is considered, a succession of emissions will generate a number of ellipses which have a common point of intersection, thereby defining the image point (Fig. 2). The image of a radiating body consists of many small elements, each representing a small area of the source. The size of the elements is, therefore, chosen so that there are sufficient emissions from each to produce a statistically stable image. The simplest image which can be formed is by direct summation of all the ellipses produced. Generation of each ellipse involves the computation of coordinates, $\mathbf{x}$ from the equation relating the scalar product of the two vectors, $(\mathbf{x}_1 - \mathbf{x}_2)$ and $(\mathbf{x} - \mathbf{x}_1)$, to the scatter angle, α . For convenience this equation is written as

$$\cos \alpha = \overline{(\mathbf{x}_1 - \mathbf{x}_2)} \cdot \overline{(\mathbf{x} - \mathbf{x}_1)} \quad (2)$$

where a bar denotes a unit vector.

Vector $\mathbf{x}$ must also be constrained to lie in the image plane defined by

$$\mathbf{a} \cdot \mathbf{x} = b \quad (3)$$

The image plane is defined only by the parameters, a , and b , in equation (3), and so the choice of the depth and inclination of the plane is arbitrary. It is possible to project images on to a multitude of planes using the same set of measurements, and even to superimpose them to obtain three-dimensional images.

Figure 3 shows a schematic diagram of the proposed imaging system. There will be errors because of noise in the amplifiers, and because of quantisation and round-off into the digital processor. The basic source of error is, however, the detector, which is analogous to the lens in a normal optical system. As it is envisaged that the system will be able to cope with changes in the source distribution, the dynamic aspects of these errors are of considerable importance in relation to the design of the components.

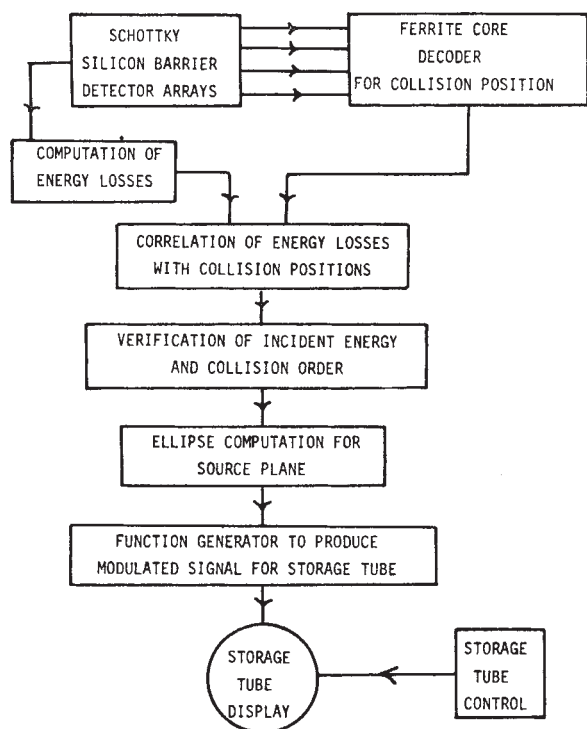


Fig. 3 Schematic representation of the proposed system.

The detector must be capable of measuring the coordinates and intensity of an interaction, and liquid or solid scintillators, proportional chambers and semiconductor detectors would be suitable. Energy resolution, response time and transmission properties suggest, however, that the latter offers the best solution and a prototype is under construction, based on a lattice of Schottky barrier orthogonal arrays of 0.5 mm cubic elements. Because the device uses Compton scattering, it is not necessary to use high atomic number detectors working at very low temperatures.

It is normal in image processing to define the optical accuracy in terms of a point spread function; a complete image is the result of convolution with source distribution. In collimator systems there is a one-to-one correspondence between source

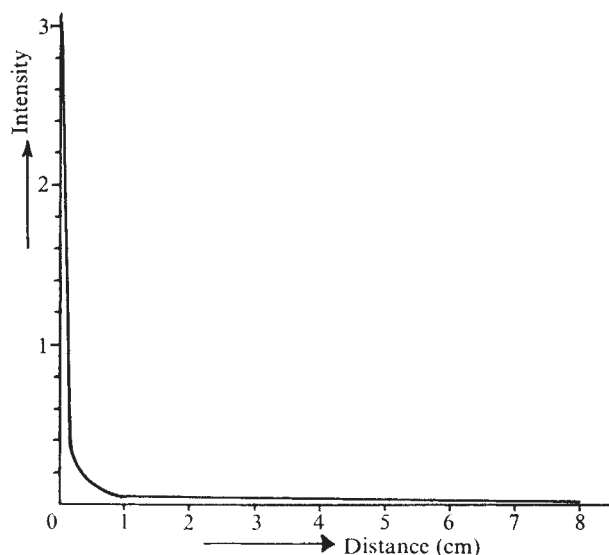


Fig. 4 Point spread function of the Compton camera.

points and a mean point on the image plane. The point-spread function gives the statistical spread about this mean. In the proposed system a source point generates a deterministic ellipse on the image plane, in the absence of measurement errors. It is possible to determine the statistical spread, resulting from measurement errors, about the ellipse. This distribution is not, however, directly useful in an assessment of image formation and, in practice, each image point is obtained by an accumulation of ellipses. It is therefore more appropriate to define a point spread function for the sum of ellipses about their common point of intersection. Figure 4 shows the theoretical point spread function assuming the source to be 10 cm from the detector. This is a more pessimistic estimate than the so-called intrinsic point spread function.

The point spread function used here assumes statistical stability. That is, the addition of further ellipses does not improve the function. Although this results in a sharp distribution, it is fair because comparisons with other systems allow consideration of the number of counts necessary to form an image. In more conventional measurement terms this is concerned with the relationship between signal : noise ratio and the integration time. To achieve this evaluation in a meaningful way a uniform source sheet with a small hole at its centre is considered (Fig. 5). The image contours will not have a discontinuity corresponding to the hole edge but will vary gradually to a minimal value at the centre of the hole. Detection of the hole therefore depends on the fractional image density difference between the outside and the interior of the hole (Fig. 5).

The other significant difference between the proposed system and the collimator system is that data processing is an integral part of the optical system. In the Compton system it is possible to effectively improve the optical properties of the detector by

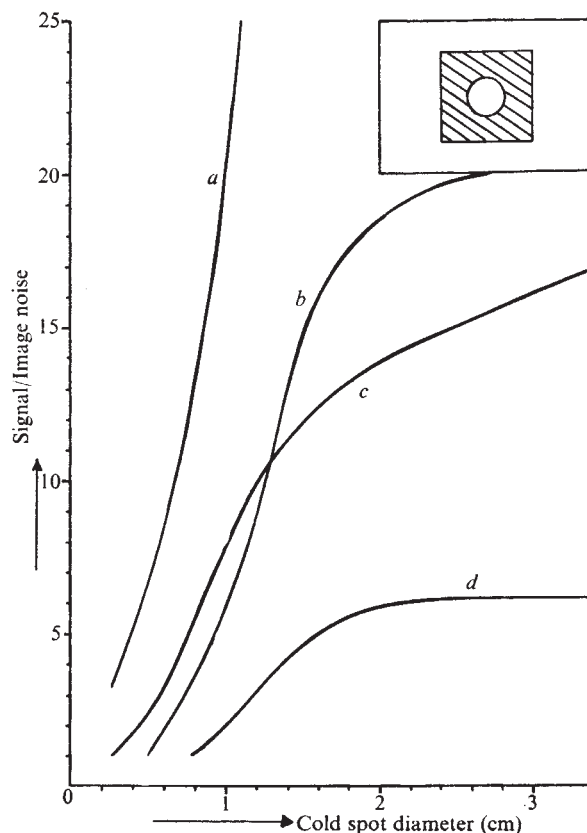


Fig. 5 Signal to image noise ratio against the cold spot diameter. *a*, Compton system, 10^6 ellipses (10 s); *b*, collimated system, 10^6 counts (1,000 s); *c*, Compton system, 10^5 ellipses (1 s); *d*, collimated system, 10^5 counts (100 s). Imaging times refer to a 400 Ci source; average source activity is one third of peak activity; there was no image processing. Inset: radioactive source sheet with a hole at its centre.

operating on the incoming data during the formation of the image. In the collimator systems, data processing is normally only applied to the completed image.

If it were necessary to draw each individual ellipse the display problem would be immense. Using statistical estimation, however, it seems possible to produce best estimates of the intersection point without having to display each ellipse. The extra computation involved is extremely small.

A number of practical problems arise which have led to further studies. These include the determination of the sequence of interactions, the removal of spurious data by time coincidence methods, amplification of a large number of low intensity pulses, and detector fabrication. Research carried out so far indicates that none of these problems have any serious implication to the basic concept.

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Carbon isotope fractionations in natural gases

THE origin of gaseous hydrocarbons has been considerably illuminated by isotope analyses of natural gases. We shall try here to establish a model, explaining the isotopic fractionations of methane as a function of the thermocatalytic development of the corresponding source material. Because the origin of gaseous hydrocarbons is closely related to the formation of crude oil on one hand and the thermocatalytic state of the organic source material on the other, isotopic investigations can be of great interest in exploration for hydrocarbons.

The results of isotope analyses of natural gases from Japan¹, Taiwan², Italy³ and the DDR⁴ are compared with isotope measurements of gases from the Federal Republic of Germany⁵.

The German gases were taken from different geological formations and wells of the north-western German oil and gas province. The methane (C_1) of the sampled gases was separated from the remaining gas components by liquid oxygen separation⁵, combusted to carbon dioxide and its carbon isotope composition determined in a mass spectrometer (Atlas-Varian M 86). The isotope ratios are given in the usual δ notation:

$$\delta^{13}C_1 = \frac{[(^{13}C/^{12}C)_{\text{sample}} - (^{13}C/^{12}C)_{\text{standard}}]}{(^{13}C/^{12}C)_{\text{standard}} \times 1000(\text{‰})}$$

The reference gas (standard) is the PDB-standard, which is representative of the isotopic composition of a marine carbonate. The reproducibility of the δ values is $\pm 0.3\text{‰}$. The $\delta^{13}C_1$ values of dry natural gases are shown in Fig. 1 and plotted against the ratio of methane (C_1) over all saturated gaseous hydrocarbons (ΣC_n). Gases associated with oil or with high carbon dioxide content are omitted because of possible isotope exchange fractionations.

Biogenic alteration of the organic matter is predominant during the first few feet of sedimentary burial. Bacterial action on the organic material results in methane enriched in the light ^{13}C isotope⁶. Further sedimentation brings the organic material

to a greater depth and thus into higher temperature zones. Chemical processes such as hydrolysis, cracking and hydrogen disproportion become more and more important. Reaction rates double for every $10^\circ C$ rise in temperature. Isotopic fractionation occurs as a result of the different binding energies of the various isotopic molecules: ^{12}C – ^{12}C bonds are the first bonds to be destroyed by thermal stress⁷. The methane emerging from the first stages of thermocatalytic alteration of source material is therefore rich in the light isotopes. Consequently the 'young' gases from Japan and Southern Italy (Fig. 1) are characterised by large amounts of methane ($C_1/\Sigma C_n > 0.99$) with low concentrations of ^{13}C ($\delta^{13}C_1 < -60\text{‰}$). With deeper burial, as the thermal stress on carbon complexes increases, more ^{13}C – ^{12}C bonds are broken and the gas becomes isotopically heavier. Increasing thermocatalysis also causes the ratio of methane to all saturated gaseous hydrocarbons ($C_1/\Sigma C_n$) to change in favour of the higher hydrocarbons⁸. The extent of thermocatalytic change is dependent on temperature, that is, on the depth of the source bed formation, the time of maximum temperature influence, and the time when the reservoir rock became a closed system. Therefore, the degree of thermocatalytic alteration of organic material is sometimes linked to the geological age of the source bed formation and, subsequently, to the age or depth of the reservoir bed of the natural gases. This trend is shown by the data of the Quaternary Japanese, the Tertiary Southern Italian and the Permian or Triassic natural gases from the Thuringian Basin (Fig. 1), in which the source bed material is of predominantly marine origin. A turning point in the development of marine organic material is represented by the data on Permian natural gases from the Thuringian Basin, ($C_1/\Sigma C_n \approx 0.8$; $\delta^{13}C_1 \approx -42\text{‰}$). As the organic source material is further altered, the hydrocarbons released

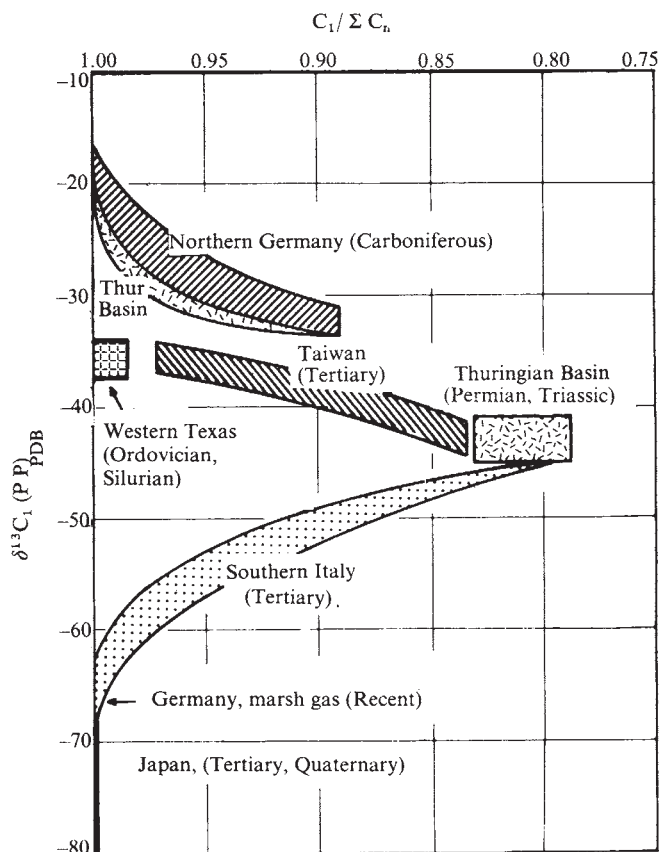


Fig. 1 Isotopic variations in methane in natural gases (refs 1–5, 9 and W. Stahl and B. D. Carey, in preparation).

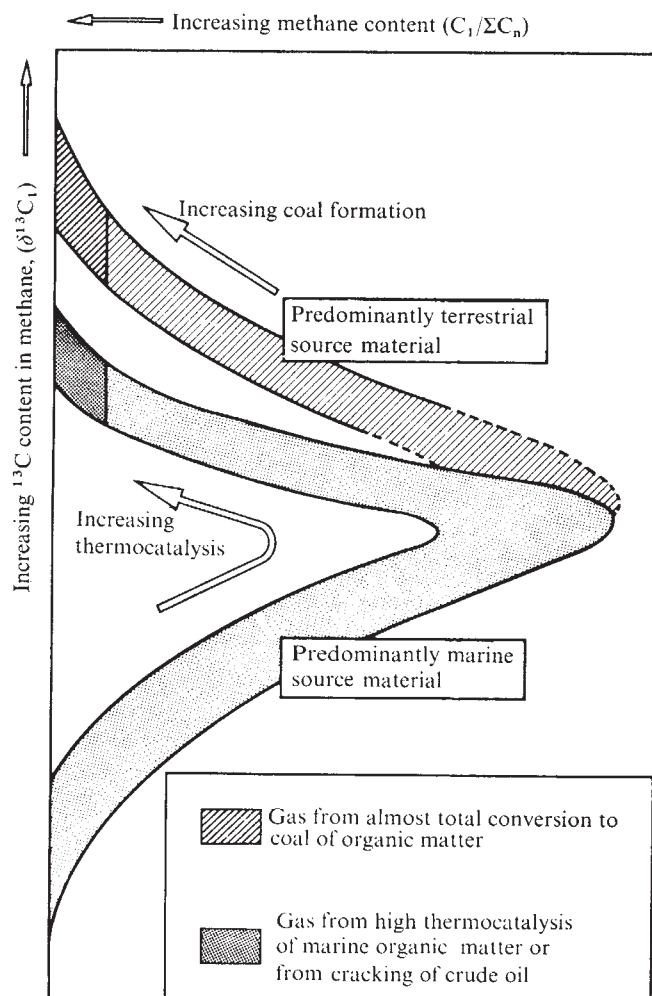


Fig. 2 Fractionation model.

are increasingly cracked, and the methane content of the gas and the $^{13}\text{C}/^{12}\text{C}$ ratio of the methane increases. This stage is represented by the Taiwan data. The thermocatalytic development of this source material has been accelerated by magmatic events during the Tertiary. In the final stage, all higher hydrocarbons are converted into methane by cracking, enriched in the heavy isotope. This final stage of thermocatalysis is shown by the analytical data of deep dry Ordovician and Silurian gases from West Texas (W. Stahl and B. D. Carey, in preparation).

On the other hand, the methane content of the gases produced during the formation of coal, for example the Carboniferous, Permian and Triassic natural gases of the Federal Republic of Germany, is also considerably enriched in ^{13}C . These gases, formed when predominantly terrestrial organic material is converted to coal, show properties comparable with gases from the cracking stage of marine source material: the $\delta^{13}\text{C}_1$ values change with increasing thermal stress in favour of the ^{13}C isotope, methane content increases, and the amount of higher hydrocarbons decreases with increasing degrees of conversion to coal.

The observations discussed here are summarised in Fig. 2, where an economically important application of the model is demonstrated, the distinction of pure methane gases of different origin. The origin of methane gases characterised by low δ values, that is enriched in ^{12}C , can be traced back to young organic material, which was only affected by relatively low temperatures. High ^{13}C concentrations in methane show that the gas originated during coal formation processes or during the thermal destruction of marine organic matter or liquid hydrocarbons under relatively high temperatures. In

this case, the probability of finding crude oil will be extremely small.

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Evidence on the locomotor pattern of *Homo* from early Pleistocene of Kenya

HERE I present the results of the application of two multivariate statistical techniques, a linear function and a distance statistic, in the analysis of a hominid talus bone. They provide additional objective evidence that this talus, found at East Rudolf, North Kenya, in 1971, should be included in the genus *Homo*. They also indicate that the morphology of the bone is compatible with early *Homo* having a lower limb functioning like that of modern man.

The specimen, KNM-ER813 (ref. 1), is a well preserved right talus; details of its morphology have been described and illustrated elsewhere². It was recovered from beds between tuffaceous horizons that have been correlated with tuffs dated at 2.61 ± 0.26 and 1.57 ± 0.00 million yr (Myr) by argon isotope techniques³.

Comparative data which have been used as a reference framework are listed in Table 2 and details of the groups have been given in previous reports^{4,5}. Three angular and five linear measurements were made on 318 adult tali. These measurements relate to the size and disposition of the articular surfaces, and were selected both for their reproducibility and for their biomechanical significance in locomotion. The five linear measurements were made up into four indices to reduce the effects of overall size disparity in bones that are of the same shape. The values of the variables for the fossil are given in Table 1 (see ref. 4 for further details of measurements).

Canonical analysis of these data, using the BMD 07M program⁶, resulted in five significant canonical variates. Variate I, containing 76% of the total variability, isolated the modern human tali; variate II separated the knuckle-walking category; the two subsequent variates separated the arboreal forms, from the rest (III), and from each other (IV); variate V was nondiscriminatory.

The canonical coefficients of these first four variates have been applied to the fossil data, a procedure that is quite proper⁷. In all the variates, the fossil is aligned with the modern human tali and in the first canonical axis (which by definition effects the greatest measure of separation) the fossil is especially close to man (Fig. 1).

Table 1 Data for the fossil talus

Variables	Horizontal angle	Dip angle	Torsion angle	Neck length index	Head projection index	Trochlear shape index	Fibular facet projection index
KNM-ER813	14	25.5	48	130	40	91	222

The Mahalanobis D^2 distances of the fossil from the comparative groups are given in Table 2. The distances between the fossil and all three groups of modern human tali are small, so small in the cases of the Bushmen and the Modern groups that the distances are not statistically significant. These D^2 results indicate that the behaviour of the fossil in the canonical variates, in particular in variate I, are a true reflection of its affinities (Oxnard has recently pointed out that this may not be the case where large and significant D^2 distances are involved⁸).

Although the multivariate analysis results are expressed in fewer dimensions than there are measurements, they confirm the close morphological similarity that exists between the fossil and modern human tali. Their relevance for its classification are that they strengthen preliminary assessments which placed this fossil talus in the genus *Homo*^{1,2}. There is evidence from other fossils that the *Homo* lineage is present at East Rudolf in deposits both older and younger than those in which this fossil was found^{1,9}. It cannot be certain to which grade or chronospecies of *Homo* this talus belongs for no talus has been found associated with fossils characteristic of the 'erectus' grade of the *Homo* lineage. Two femur shafts, however, KNM-ER737 and 803, that are broadly contemporaneous with the talus, show features characteristic of the *Homo erectus* femurs from Choukoutient and Olduvai Gorge¹⁰.

Considering the locomotor implications of these results, although the modern human locomotor pattern seems to be associated with a particular talar morphology, one would be guilty of the *post-hoc* fallacy in assuming that a similar talus was necessarily part of the same sort of hind limb functional complex. Nevertheless, with that caveat, the functional implications of the canonical analysis results, combined with the close morphological affinity of the fossil talus with the modern human bones, make it probable that the locomotor pattern of this early hominid was like that of modern man. More comparative and functional data from other bones in the lower limb are needed to confirm this hypothesis.

One of the objects of accumulating comparative material has been that the groups could be used as a framework to compare fossil bones. Similar fossils would be associated on the canonical variates and their D^2 distances from the

comparative groups should be similar. It is of particular interest, therefore, that this East Rudolf talus and a talus of a similar time period, OH8 from Olduvai Gorge, Tanzania, behave differently in the analysis⁴. The suggested implications of these differences for the classification and phylogenetic interpretation of the Olduvai specimen will be presented elsewhere.

Table 2 D^2 distances and probability levels between special case and groups

No.	Group	
1	<i>Homo sapiens</i> (Modern)	14.6 5.0%
2	<i>Homo sapiens</i> (Anglo-Saxon)	21.8 <0.005%
3	<i>Homo sapiens</i> (Bushman)	11.5 10.0%
4	<i>Gorilla</i>	56.4 <0.005%
5	<i>Pan</i>	57.6 <0.005%
6	<i>Papio</i>	76.45 <0.005%
7	<i>Cercopithecus</i>	67.6 <0.005%
8	<i>Colobus</i>	63.6 <0.005%
9	<i>Pongo</i>	88.1 <0.005%

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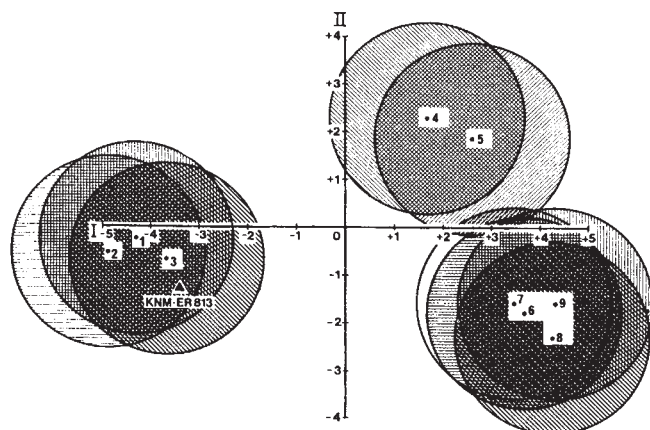


Fig. 1 Plot of canonical variates I and II. Circles are drawn with a radius of 2 s.d. The group numbers are identified in Table 2.

Coal smoke and mortality of the elderly

MORTALITY from falls in old people is so closely related to mortality from fractures of long bones, particularly of the proximal femur, that it seems to be related to bone fragility¹⁻³. Although the mortality of women aged 75 yr and over is more than 50% higher than that of old men, there has been a close relationship between male and female annual mortalities in both Britain and the United States. This suggests that some common contemporary factors affected the mortality, and possibly the bone fragility, of each sex, even though their total experiences

of mortality were substantially different. The evidence presented here suggests that coal smoke is one such factor.

A striking feature of mortality from falls of the elderly in Britain has been the recession in mortality over the period of wartime and post-war austerity from 1940 to 1950, subsequently rising to be followed later by a steady fall since 1958–59. This pattern, which appears in the mortalities of both men and women aged 75 yr and more^{1,2}, resembles that shown by annual estimates of atmospheric pollution by domestic coal smoke. This is illustrated in Fig. 1.

The estimates of annual emission of smoke from house coal shown in Fig. 1 were obtained, together with estimates of railway smoke from steam locomotives and industrial smoke, from estimates given by the Warren Spring Laboratory⁴ for the years 1952–65 based on data of coal consumption. For years before and after that period the factors used to make these estimates (weight of smoke = 3.5% of domestic coal, 2% railway coal, 0.5–1.2% industrial coal) were applied to data of annual coal consumption compiled for those years⁵. The relationships of mortality to estimated annual emission of coal smoke are shown in Table 1. Mortalities are related to domestic smoke pollution for both men and women but only for bronchitis in women to industrial smoke; the highest correlations being between domestic smoke and falls, both in men ($r = 0.73$) and in women ($r = 0.82$).

The immediate cause of death of an elderly person immobilised by a fractured femur is usually a lung infection. The mortality of middle-aged people up to the age of 75 yr in County Boroughs has been related, *inter alia*, to estimates of domestic smoke pollution in the different boroughs, a principal cause of the relationship being mortality from bronchitis^{6,7}.

The mortality from bronchitis of women aged over 75 yr is shown in Table 1 to be highly correlated with mortality from falls ($r = 0.70$) and with domestic smoke ($r = 0.78$); bronchitis in men has lower correlation coefficients both with falls ($r = 0.51$) and smoke ($r = 0.37$). Mortality of men from bronchitis is higher than that of women, and additional factors, particularly tobacco, affect their mortality from bronchitis. The marked recession in mortality from falls in 1940–50 followed by post-war peaks in 1956 and 1958–59 did not occur in mortality from bronchitis, but the annual fluctuations of mortality from bronchitis were reduplicated at a lower amplitude by the mortality from falls. There was an increased risk of death following a fall in bad years for bronchitis.

Table 1 Correlations and multiple regressions of death rates from falls with death rates from bronchitis and domestic smoke pollution

Coefficients of linear correlation (r)		Bronchitis mortality	Domestic smoke	Industrial smoke
		1	11	111
		r	r	r
Bronchitis mortality	male		0.37*	0.17†
	female		0.78†	0.63†
Falls mortality	male	0.51*	0.73†	0.12†
	female	0.70†	0.82†	0.17†
Multiple regression				
Partial regression coefficients (b)		b_1	b_{11}	
Falls mortality	male	0.046*	0.479†	
	female	0.024†	0.721†	

For the multiple regression the multiple correlation coefficient, R was 0.78† for men and 0.83† for women.

Industrial smoke includes railway smoke.

* $P < 0.05$.

† $P < 0.001$.

‡ Not significant.

Analysis by multiple regression with mortality from falls as the dependent variable and two independent variables—domestic smoke pollution and mortality due to bronchitis—gives highly significant regression coefficients for smoke pollution both in men and women ($P < 0.001$); the regression coefficient for bronchitis for women is not statistically sig-

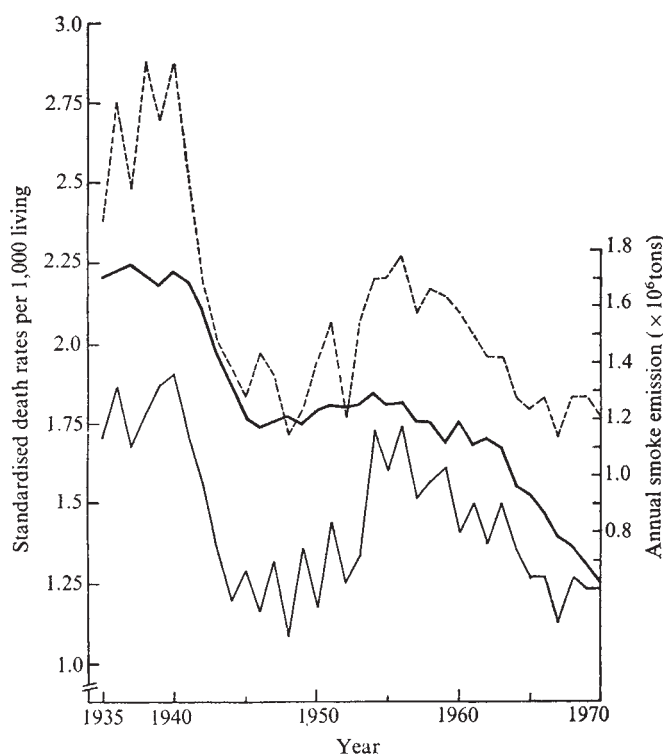


Fig. 1 Death rates at age 75 yr and over for accidental falls (E880-7: eighth revision International Classification of Diseases) compared with estimated annual emission of domestic coal smoke. Death rates are directly standardised for age and sex on the total male and female 1961 population at age. — male death rates; --- female death rates; — smoke emission.

nificant ($0.25 < P < 0.4$) but is probably significant for men ($P = 0.02$) at a much lower significance than for smoke pollution. Mortality from falls (and by inference from fractures of the long bones) seems, therefore, to be related to domestic smoke pollution independently of the relationship to mortality from bronchitis.

The steep rise in femoral fractures with advancing age in Britain may be attributable to an absolute or relative deficiency of vitamin D (refs 8 and 9). In Detroit (latitude 42°N) serum antirachitic activity, as well as being related to season and sunshine, was significantly less in symptomatic osteoporotic subjects than in the skeletally normal¹⁰. There is increasing evidence of the importance of endogenous synthesis of vitamin D by ultraviolet irradiation of 7-dehydrocholesterol in the skin¹¹, even in the higher latitude of London (52°N) where there are seasonal changes in plasma levels of 25-hydroxy-cholecalciferol¹². When smoke pollution was at its height in 1937–39, it was estimated that in Leicester (latitude 53°N) at least 30% more ultraviolet daylight would have reached the centre of the city in winter if all the smoke in the atmosphere had been eliminated, and occasionally the loss was more than half¹³. The effect of smoke on mortality from falls and fractures may therefore have been due to reduced endogenous synthesis by ultraviolet light.

These relationships between mortality and environmental change have implications beyond the restricted area of geriatric mortality. Because they are a dying generation at the end of the human life span, factors affecting the inevitably high mortality and causes of death of people over 75 yr of age are fewer and simpler than the much more complex influences affecting the lower mortalities of middle age. Though they will succumb to illnesses and infections which would be trivial and easily treated in a younger person, they will have survived or no longer be subjected to many of the stresses and influences competing for life or death affecting mortality and morbidity in middle

age. They may therefore show relationships of mortality to single factors in environmental change which are masked by complex multifactorial influences at younger ages. The simpler relationship of mortality from bronchitis in women to both domestic and industrial smoke is particularly notable.

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Genetics of cannibalism in a viviparous fish and its relationship to population density

THE success of viviparous species is a result of their capacity to produce small numbers of advanced offspring with high survivorship potential. Cannibalism, which would appear to be antithetical to this adaptation, is widespread among viviparous fishes¹⁻³. I have examined the genetics of cannibalism and the significance of its density-dependent mechanism in teleosts of the genus *Poeciliopsis*. A five-membered unisexual-bisexual complex has evolved in this genus through hybridisation of a cannibalistic species, *Poeciliopsis monacha*, with the noncannibalistic species, *P. lucida*. Hybridisation of these two species formed *P. monacha-lucida*⁴, a diploid unisexual; backcrosses of this diploid form with each of its progenitors resulted in the evolution of two triploid unisexuals: *P. monacha-2 lucida* and *P. 2 monacha-lucida*⁵. All these rely on males of the bisexual species for fertilisation.

The unisexual-bisexual complex represents an array of morphological and biochemical characters^{3,6} that demonstrate the genetic intermediacy of unisexuals according to genome dosage of parental forms. I have investigated whether the degree of cannibalism also follows genome dosage and whether adult and/or young densities influence the expression of this trait.

Live and preserved stocks of *Poeciliopsis* were obtained from natural populations in the Rio del Fuerte of Sonora and Sinaloa, Mexico. Mature virgin females were maintained in 75.7-l aquaria with sparse amounts of *Najas* and *Vallisneria*, many snails, and fed twice daily with commercial dry food supplemented with live *Daphnia*. Females were established at densities of five, ten, fifteen and twenty, plus a control with no females.

Young fish obtained from stock females were screened for developmental abnormalities on the day of birth, and then introduced at densities of five, ten, fifteen and twenty

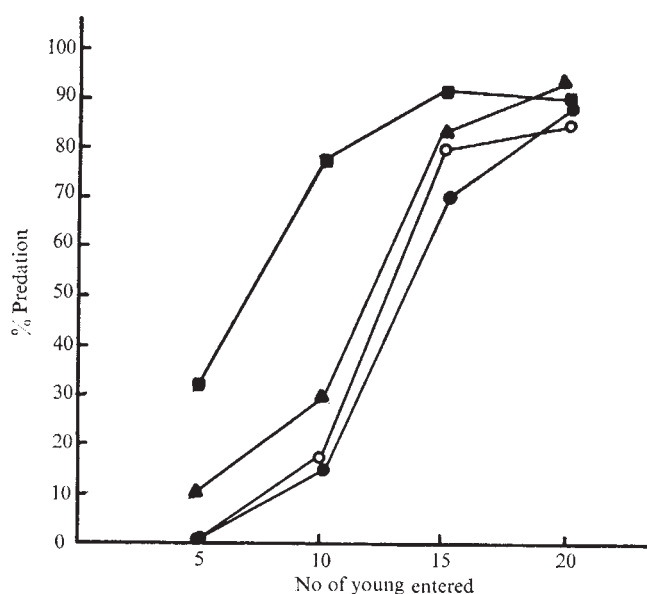


Fig. 1 Relationship of predator and prey densities to predation rates. Females of *Poeciliopsis monacha-lucida*, *P. 2 monacha-lucida* and *P. monacha* served as predators of newborn young of all five species of *Poeciliopsis*. These females were used because of large sample sizes and their consistent cannibalistic behaviour. Densities of females were: ●, five; ○, ten; ■, fifteen; ▲, twenty.

into the experimental aquaria containing the mature females. Most young were of the same brood; however, samples of fifteen and twenty young were formed occasionally by combining broods of different females. Young remained with the predators for 24 h, after which survivors were removed and counted. Preliminary experimentation demonstrated that young would not be cannibalised after 24 h. Mortality in fifty controls each of 24 h duration was zero.

Among the five unisexual-bisexual *Poeciliopsis*, *P. lucida* proved to be noncannibalistic whereas *P. monacha* consumed young in nearly all trials (Table 1). *P. monacha-lucida*, the genetically intermediate hybrid, ate young in about three out of four trials. The two triploids cannibalised at rates similar to that of the bisexual species to which they are genetically more similar: *P. monacha-2 lucida* ate young nearly as infrequently as *P. lucida*; *P. 2 monacha-lucida* nearly as avidly as *P. monacha*. The degree of cannibalism, like morphological expression, thus comprises an array directly related to genome dosage. This is the first time the predatory phenomenon has been demonstrated to be heritable. Stomach analyses of wild females confirm that certain species of *Poeciliopsis* eat young and that cannibalism is not a laboratory artefact.

Cannibalism, believed to be polygenically controlled, is a complex behavioural phenomenon related to feeding habits. The practice of cannibalism by *P. monacha* and not by *P. lucida* is probably related to their different morphology and ecology. Dentitions, for example, differ: *P. monacha* has many tricuspid inner teeth whereas *P. lucida* has few unicuspid inner teeth³. *P. monacha* is found in small head-water pools, *P. lucida* in large and stable tributaries.

Table 1 Frequencies for which any young were cannibalised by unisexual-bisexual *Poeciliopsis* in 136 trials

Species	Total no. of trials	No. of trials in which young were cannibalised	F
<i>P. lucida</i>	19	0	0.0
<i>P. monacha-2 lucida</i>	17	2	0.12
<i>P. monacha-lucida</i>	54	40	0.74
<i>P. 2 monacha-lucida</i>	25	22	0.88
<i>P. monacha</i>	21	20	0.95

Table 2 Probability model comparing total survivorship potential of young produced by fishes with superfoetation and fishes without superfoetation in a catastrophic environment

Intervals of 10 d (total 100 d)	1	2	3	4	5	6	7	8	9	10
Young production with superfoetation	10	10	10	10	10	10	10	10	10	10
Young production without superfoetation	20	0	20	0	20	0	20	0	20	0
$P_x = \left(\frac{n!}{x!(n-x)!} \right) (p^{n-x} q^x) \times (\text{No. of young}) = (\text{total young surviving})$										
$P_0 = 0.349 \times 100 = 34.9$										
$P_1 = 0.387 \times 90 = 34.83$										
$P_2 = 0.194 \times 80 = 15.52$										
$P_3 = 0.057 \times 70 = 3.99$										
$P_4 = 0.011 \times 60 = 0.66$										
$P_5 = 0.001 \times 50 = 0.05$										
$P_6 \dots 10 = 0 \times \text{No. of young} = 0$										
$P_x = \left(\frac{n!}{x!(n-x)!} \right) (p^{n-x} q^x) \times (\text{No. of young}) = (\text{total young surviving})$										
$P_0 = 0.59 \times 100 = 59.0$										
$P_1 = 0.328 \times 80 = 26.24$										
$P_2 = 0.073 \times 60 = 4.38$										
$P_3 = 0.008 \times 40 = 0.32$										
$P_4 = 0 \times 20 = 0$										
$P_5 = 0 \times 0 = 0$										
$\sum_{x=0}^{10} (P_x \times \text{No. of young}) = \text{total No. young surviving in 100 d} = 89.95$										
$\sum_{x=0}^5 (P_x \times \text{No. of young}) = \text{total No. young surviving in 100 d} = 89.94$										

The probability of a catastrophe occurring in any one day is 0.1. n is the total number of broods; x is the number of broods lost; p is the probability of a brood surviving (0.9); q is the probability of a brood lost (0.1); P_x is the probability of x number of broods being lost.

Previous experimentation with *Poecilia reticulata* on cannibalism and fecundity under crowded conditions, supported the hypothesis for a water-borne substance that acted to reduce fecundity or increase the cannibalistic response². These conclusions do not seem to be applicable to *Poeciliopsis* populations. Also, because cannibalism had previously been proposed as a density-dependent population regulatory mechanism in *Poeciliopsis*⁷, I analysed my results with respect to variable numbers of predator and prey (Fig. 1).

Intensity of predation is a function of the number of females present: the more females are present, the more young will be consumed. But more critically, intensity of predation is a function of the number of young introduced. At densities of five or ten young, predation clustered at 0–25% regardless of female density. With fifteen to twenty young, cannibalism increased to 70–90%. For 128 trials, a two-way analysis of variance was used (F statistic highly significant ($P \leq 0.001$) for young density and ($P < 0.001$) for female density).

The hypothetical value for the 'critical density' of between ten and fifteen young is consistent with laboratory observations of females preying on young. When more than ten young were introduced to aquaria containing adult females, the young tended to school or aggregate near the surface, a characteristic of newborn *Poeciliopsis*. Aggregates of young stimulate an attack response first by one female followed by mobbing behaviour from the others. After the initial attack, young quickly disperse in the vegetation or remain immobile; in the absence of schools, the searching response by the females gradually subsides over the next few hours.

Aggregates of ten young or fewer do not stimulate a predatory reaction; however, they are eaten at random when encountered by a female, thus explaining the relationship of the number of females to the rate of cannibalism. Cannibalism is less related to hunger than to predator-prey density phenomena. Females starved for 2–4 d ate no more young than did well-fed females. Furthermore, females encountering large densities of young remain unsatiated until the brood is eradicated. Discrimination of young is not involved. In a mixed environment, the probability of an individual eating its own young is low since cannibalism is suppressed until parturition is completed.

The adaptive value of cannibalism is unclear. Experimentation indicates advantage for lower densities of young. Newborn young emerging in smaller groups should have higher survivorship values than young born in larger groups.

All species of *Poeciliopsis* exhibit superfoetation, the simultaneous development within the ovary of more than one embryonic stage. *P. lucida*, *P. monacha* and associated unisexuals produce half as many young twice as often as

females of the same size of *Poecilia reticulata* which do not have superfoetation. The former have mean brood intervals of 11.6 and 10.3 d respectively, whereas the latter has 21.1 d; *P. monacha* and *P. lucida* females of 30 to 35 mm total length have mean brood sizes of 11.8 and 11.0 young respectively compared with 30–35 mm *P. reticulata* females with broods of 24.2 young⁸ (unpublished work of myself and R. J. Schultz). The birth of small broods at frequent intervals may be an adaptation to a critical density for cannibalistic predation.

Superfoetation is believed to have evolved independently in response to various selection pressures^{9–10}. Several theories on the adaptive value of superfoetation appear reasonable, but they break down under close scrutiny. Superfoetation may allow females to utilise space devoted to reproduction more efficiently by partitioning the young into different developmental states, thereby increasing total fecundity; however, fishes without superfoetation have attained brood sizes of 180 and 315 young¹¹ far exceeding twice the maximum brood size of 80 young of *P. monacha* females of comparable sizes (unpublished work of myself and R. J. Schultz).

Superfoetation may also be beneficial to fishes inhabiting unstable environments such as those of *Poeciliopsis* which are characterised by floods, droughts and extreme temperatures, all with the potential of eradicating an entire brood of young. By scattering broods of smaller numbers, young of fishes with superfoetation may exhibit higher survivorship potential. But, a simple probability model contrasts the reproductive potential of fishes with superfoetation with that of fishes without superfoetation in a catastrophic environment where catastrophes are random and unpredictable (Table 2). In this design, both types of fish have equal probability of producing the same numbers of young in a set interval: superfoetation yields no advantage.

The adaptive value of superfoetation is unclear. In spite of the function of cannibalism in the regulation of population size, the adaptiveness is also ambiguous. However, superfoetation and cannibalism are correlated in the production and survivorship of small broods in *Poeciliopsis*. Cannibalism is probably uninformed as a selective force in the evolution or maintenance of superfoetation; its persistence in viviparous fishes without superfoetation supports this. But with the presence of the numerical predator-prey mechanism, cannibalism appears to be tolerated in the evolution of natural populations when superfoetation is present as a reproductive strategy.

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Depth perception in disparity gratings

OBSERVATION of sinusoidal spatial modulation of luminance has proved a valuable tool in the study of visual processing of luminance distributions on the retina. Information on the limits of processing capability has been gained in such diverse fields as the optics of the eye¹, neurophysiology of the retina², movement^{3,4} and orientation^{5,6} sensitivity and cortical processing of spatial frequency^{7,8}. In the field of stereoscopic vision, Blakemore⁹ and Fiorentini and Maffei¹⁰ have found that when luminance gratings of slightly

different spatial frequencies were presented to each eye, an apparently tilted grating was perceived even if one monocular grating was moving quickly relative to the other. Furthermore, tilt is perceived from gratings of the same spatial frequency but differing in contrast by 50% or more. These phenomena are not easy to explain on the basis of disparities at corresponding retinal points, but seem to require more global processing of the whole image.

Julesz¹¹ has reached a similar conclusion on the basis of experiments with random-dot stereograms, which have the unique advantage of eliminating all correlated monocular cues to the stereoscopic figure (although the random dot texture acts as a monocular textural cue that the figure has a constant depth and works against the disparity information to some extent). Stereopsis can be obtained under several conditions which make it difficult to conceive of a local disparity process that could produce depth perception. For example, alternation of two stereograms at 20 Hz does not destroy perception of a stereoscopic figure¹². Furthermore, in ambiguous stereograms the depth perceived depends on the global organisation of nearby dots¹³.

It would be of value to combine the advantages of random-dot stereograms and of sinusoidal modulation techniques in the study of spatial limitations of cyclopean depth perception. I have previously studied the effects of sinusoidal modulation of binocular disparity along a vertical axis on stereoscopic perception using line stereograms¹⁴. The main stereoscopic processing limitations noted were a sharp high frequency limitation in sensitivity at about three cycles per degree (c.p.d.) and a disparity scaling effect, in which the maximum disparity that could evoke a depth percept was directly proportional to the retinal angle subtended by each cycle of the vertical modulation. Thus, the disparity limit is scaled in proportion to

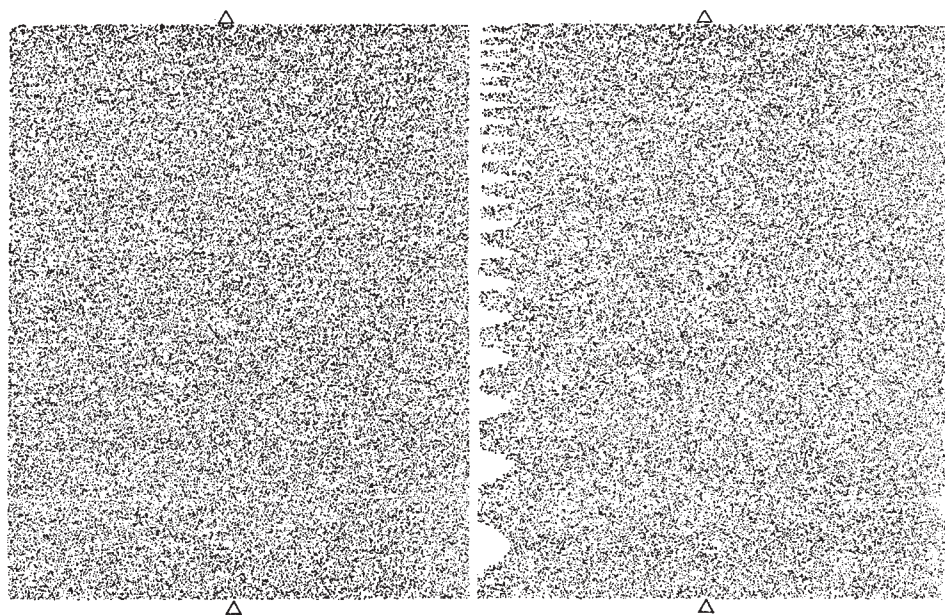


Fig. 1 Demonstration stereograting. This figure should be viewed by means of a simple stereoviewer requiring two white cards and a pair of spectacles. With the figure at arm's length hold the cards at about 10 inches from the eyes. Closing each eye in turn, align the cards so that each eye sees only one dot matrix. It should now be possible with the aid of the fixation marks to register the two matrix images as a single square. If this square remains blurred it may help to wear a pair of weakly hyperopic spectacles (in addition to any spectacles worn normally). Continued observation should reveal shallow horizontal ripples or cones increasing in spatial frequency upwards and becoming shallower towards the right. Generation of random-dot disparity gratings was achieved by a Fortran program controlling the position of randomly selected points in a 10×8 inch matrix on a Calcomp plotter. In measuring stereoscopic thresholds the problem that dot resolution is about 1 arc min visual angle, whereas disparity resolution requires down to 10 arc s, was solved by modifying the concept of a completely random matrix so that dot position was specified to one tenth of a dot width. This gave minimum possible disparity shifts of about 13 arc s at the viewing distance of 200 cm. Although horizontal changes in disparity theoretically introduce slight monocular cues to the disparity information, such cues are negligible in practice, since increasing the disparity range to ten times the largest used in this study did not introduce detectable monocular variation in dot density.

size of the modulation cycles. The experiments reported here show that the line stereogram results are replicated using random-dot stereogratings as stimuli and are probably general properties of the stereoscopic system.

Generation of random-dot disparity gratings with a large range of disparity variation required a slight modification of Julesz's concept¹¹ (see Fig. 1). A second technique enabled threshold sensitivity to be measured over a great range of amplitudes and frequencies of disparity modulation without generating a vast number of individual stereograms. By analogy with the demonstration stimulus for luminance grating sensitivity¹⁵ it is possible to produce continuous variations of both amplitude of disparity and spatial frequency as a frequency-swept, amplitude-decayed stereograting (Fig. 1). The boundary between perception of the flat random-dot texture and the rippled surface of the stereograting is determined by the spatial frequency limits of disparity processing in the visual cortex.

For demonstration, the monocular cues of the disparity ripple at the edges of the right hand matrix is present, but these were masked to straight edges during quantitative experiments. The edge ripple in relation to the straight edge of the left-hand matrix indicates the precise disparity present down each edge of the figure. Readers may verify that no changes in texture density correlated with the disparity variations are evident in Fig. 1.

To ensure that maximal stereoscopic information was available, free eye movements and long and active viewing times were encouraged. The stereogratings were viewed through a Smith orthorater stereoscope. Each subject was fully optically corrected, since individual dots were close to the limit of visual resolution. The stimuli were Xerox copies of the Calcomp plot which had a luminance of 3 cd m⁻² and were viewed at a distance of 200 cm. Subjects were instructed to concentrate on each bar indicated in turn, and decide where in the matrix the bar disappeared into a flat surface. They then directed the experimenter in marking the point on the matrix with a red mark invisible to the subject. A complete set of spatial frequencies was measured twice.

The results of the measurements of spatial frequency limitations of stereosensitivity for two subjects (Fig. 2) are in general agreement with line stereosensitivity limits¹⁴. The maximum frequency for which the stereograting can be resolved (Fig. 2a) is about 4 c.p.d. (compared with 3 c.p.d. in line stereograms¹⁴), and is only slightly affected by a tenfold reduction in dot density. (Note that at this frequency there are 7 dot widths or 70 dot positions per cycle.) The slight discrepancy between the uppermost points in Fig. 2a and the lowermost in Fig. 2b is probably due to measurement error and may also be influenced by edge effects at the edge of the grating.

Similar results may be obtained for previously published stereograms containing both vertical and horizontal gratings¹⁶. The grating becomes invisible as viewing distance is increased so that the grating subtends about 4 c.p.d. This is not due to lack of acuity for the monocular textural information, since in stereograms of the same texture at the same viewing distance a large stereoscopic figure such as a square is readily visible.

High spatial frequency sensitivity may also be studied by removing high or low spatial frequency information from one matrix of a stereogram¹⁶. Once again stereopsis is difficult (impossible for all five observers I tested) when there are no low spatial frequency cues to the stereoscopic figure, whereas lack of high spatial frequency information hardly affects stereoscopic perception.

As with all measures of stereolimits there is a lower and an upper disparity for which perception of depth falls to threshold. The former will be called stereoacuity and the latter the upper depth limit. Stereoacuity falls in the region of the resolution of the Calcomp plotter, so it could not

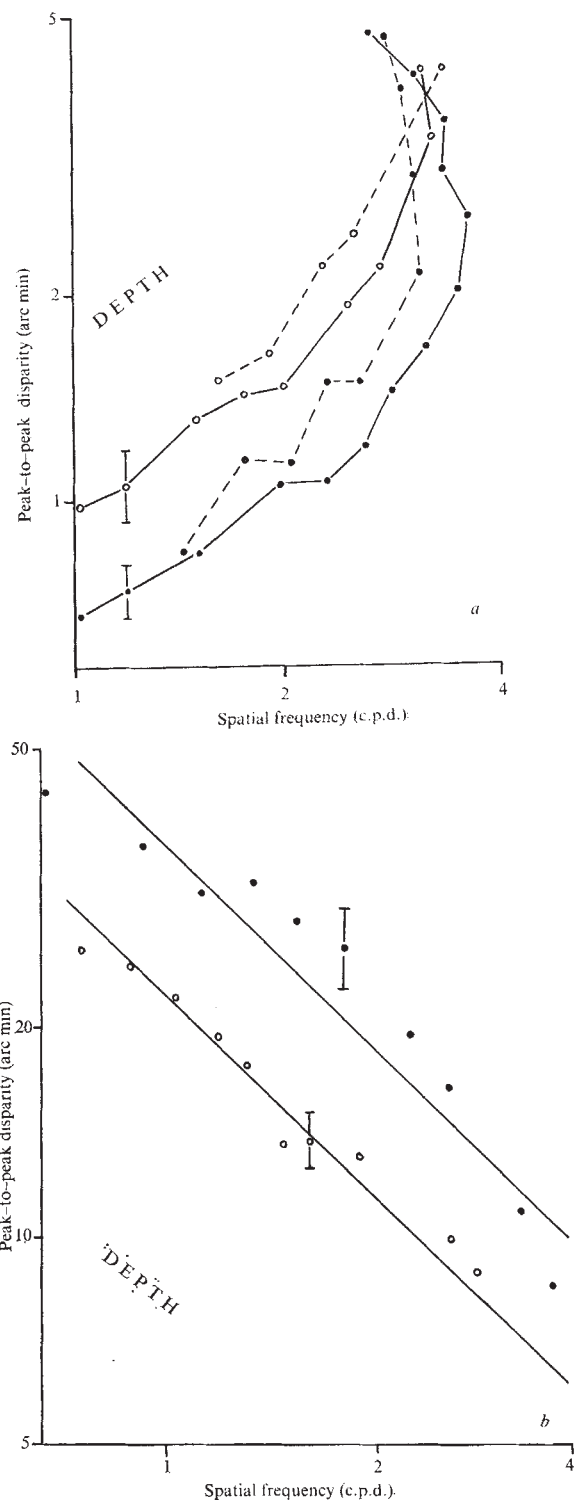


Fig. 2 Stereosensitivity for two subjects, J. Z. (○) and C.W.T. (●). *a*, Region of maximum frequency, plotted in terms of peak-to-peak disparity variation versus spatial frequency of sinusoidal stereograting. —, 40% dot density; ---, 4% dot density. *b*, Region of maximum disparity in same coordinates, showing conformity to disparity scaling effect. To obtain stereogratings with sufficiently low rate of change over a full range of spatial frequencies and amplitudes the gratings used were, *a*, 1–10 c.p.d., 0.5–5 arc min and *b*, 0.75–7.5 c.p.d., 5–50 arc min in spatial frequency and peak-to-peak amplitude range. Both these stereogratings were produced with a dot density of 40% by area, but as a check on the effect of dot density *a* was also produced with dot density of 4%. Variability of the data is shown as the average standard deviation of all pairs of readings (vertical bar above and below one point in Fig. 2). In regions where the function is vertical the bar represents horizontal variability, since the direct viewing technique does not constrain the variability to one dimension.

be measured by the present method. For the upper depth limit, the maximum disparity (Fig. 2b) decreases with increasing frequency, corresponding to a disparity scaling effect which conforms reasonably well with the equation (solid line in Fig. 2b):

$$\eta = a/\nu$$

where η is peak-to-peak binocular disparity in arc degrees, ν is spatial frequency in c.p.d. and a is a scaling constant that has the values of approximately 0.3 for J.Z. and 0.6 for C.W.T. These values may be compared with values of about 0.5 for C.W.T. and 0.33 for another subject using line stereograms¹⁴.

This disparity scaling equation describes a limit in rate of change of disparity across the retina. This limit applies equally to global stereopsis from random-dot stereograms and qualitative stereopsis from line stereograms¹⁸. The similarity of the scaling constant a for the two types of stimuli suggests that the disparity scaling limit is determined by the arrangement of disparity detectors in cyclopean space rather than by the difference in orientation between monocular line segments¹⁷.

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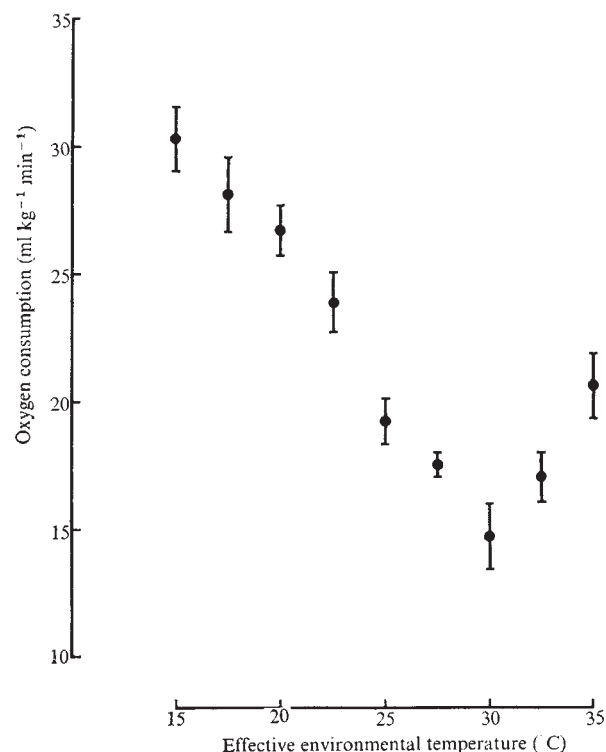


Fig. 1 Mean ($\pm$ s.e.) oxygen consumption of groups of neonatal beagle dogs (3 d old) segregated at selected environmental temperatures for 30 min.

of the term homeothermy. Body temperature stability is the product of a number of behavioural, physiological and metabolic processes^{3,6} which characterise the homeothermic state and these clearly differentiate it from poikilothermy. Appreciation of the differences is of some importance in the dog as the newborn of this species is often the subject of intensive medical care requiring segregation in an environment which makes minimal metabolic demands.

Beagle puppies were used in this study. They were housed with the bitch in insulated whelping boxes⁷ in a constant ambient temperature of 28°C. The core temperature of a post-prandial puppy 3 d old was recorded and it was then subjected to an interval of 30 min in an incubator at 30°C to establish equilibrium between core and surface temperatures before a period of experimental observation in an environmental chamber. Nine temperatures, lying between 15°C and 35°C were used, and puppies were randomly allocated to not more than two of these temperatures on the day of observation.

The environmental chamber was a Perspex cylinder surrounded by a water jacket which was incorporated into a closed air circuit, a modification of a design described earlier by other workers⁸. A puppy in the chamber lay inside a plastic cylinder bearing multiple perforations to prevent bodily contact with the wall of the chamber. The experimental environment was controlled in terms of air humidity (40-60%), air velocity (of the order of 1.5 cm s⁻¹ surface speed) and temperature. Internal wall and air temperatures were monitored by means of thermistors and the effective environmental temperature of the chamber was taken as their mean. A period of 4 min was sufficient for equilibration of the apparatus after a puppy was introduced and thereafter oxygen consumption was recorded for a period of 30 min. Carbon dioxide within the closed circuit was totally absorbed in a container of soda lime placed distal to the chamber in the direction of air flow. Depletion of closed-circuit volume was restored by addition of fixed volume aliquots (20 ml) of pure oxygen from a float recorder which refilled automatically when empty. Rate of oxygen consumption was computed as the regression coefficient of the equation relating the fixed volume of oxygen consumed to recorded times and was expressed as ml min⁻¹ dry gas at s.t.p. divided by body weight (kg). Core tem-

The homeothermic status of the neonatal dog

THE capacity of a warm blooded animal to increase production and conservation of body heat when it is cooled and to dissipate heat when it is warmed largely determines that range of environmental temperatures within which a stable deep body temperature can be successfully achieved.

In these respects the mature dog is an example of an efficient homeotherm, able to withstand the short-term effects of ambient temperatures as low as -100°C and as high as 42°C¹⁻³. In contrast, the neonatal dog demonstrates a relatively poor body temperature stability and when isolated in a cooling environment it tends to adopt the temperature of that environment^{2,4}. This has led to an assumption that the dog is poikilothermic for some time after birth^{2,5}. The concept of poikilothermy in this context may have been based on a too literal interpretation

peratures were recorded before and after each exposure by means of a thermistor probe inserted into the colon for a distance of approximately 6 cm. Puppies were observed visually and their behaviour was recorded during periods of study. Other beagle dogs 2 d old were subjected to periods of exposure in ambient temperatures between 8° and 35° C for intervals of up to 120 min. Core temperatures were recorded in these instances, but not oxygen consumption.

Metabolic activity, expressed as oxygen consumption, of 3 d old puppies which were segregated in selected effective environmental temperatures for intervals of 30 min is illustrated in Fig. 1. Mean oxygen consumption was minimal at an environmental temperature of 30° C. Lower and higher temperatures resulted in an increase in oxygen consumption. A significant difference was observed at 27.5° C ($P < 0.05$) and the mean rise of oxygen consumption over that range of cooling temperatures which was chosen was related to the degree of cooling stress. At 15° C the mean oxygen consumption was twice that which was recorded at 30° C.

Mean body weight of the group exposed to 30° C was 0.328 kg. Mean oxygen consumption was 14.7 ml kg⁻¹ min⁻¹. Assuming that 1 ml of oxygen min⁻¹ is equivalent to heat production of 7.2 kcalories/24 h (ref. 8), and using a factor of 4.184 to convert kcalories to kJ (ref. 6), the mean resting metabolic rate of these puppies in an environmental temperature of 30° C was $14.7 \times 0.328 \times 7.2 \times 4.184 = 145$ kJ per 24 h. This compares favourably with a value of 127 kJ per 24 h which can be calculated for mammals of the same weight using the generally accepted level of 293 kJ kg^{-3/4} per 24 h⁻¹ for mean standard metabolic rate^{8,9}.

Characteristic behaviour was also observed (Table 1). At the environmental temperature at which oxygen consumption was minimal, puppies 3 d old relaxed at once and remained completely unconcerned with their surroundings. At lower temperatures, a proportion of each group became alert, restless, hyperpnoeic and vocal. The degree of persistence of these responses and the proportion of animals involved increased as environmental temperatures declined. A similar syndrome was observed at temperatures of 32.5° C and higher. Both syndromes ceased at once and the puppies resumed a state of relaxation and somnolence when they were returned to an incubator at a temperature of 30° C which appeared to be the preferred environment of puppies of this age.

Core temperatures of puppies 3 d old were found on removal from the bitch to be 37.2 ± 0.4 ° C (mean $\pm$ s.d. : $n = 90$). No significant differences were recorded after isolation and exposure to 30° C in an incubator or in the environmental chamber.

Changes in the deep colonic temperature of these puppies are illustrated in Fig. 2. If it is accepted that a mean change of colonic temperature of less than 0.5° C over a period of 30 min is indicative of thermoregulation, then puppies 3 d old were able to regulate in environmental temperatures between 20° – 30° C. Exposure to temperatures outside this range resulted in deviations in body temperature which stimulated behaviour indicating that the onset of hypothermia or hyperthermia was not accepted passively (Table 1).

The influence of environmental temperatures on deep colonic temperature of puppies 2 d old after exposure for longer periods is shown in Table 2. Again, no significant changes in colonic temperatures were observed in the group exposed to 30° C in spite of a temperature differential between core and environment of 7° C. A temperature differential of 12.5° C, that is an environmental temperature of 25° C, was also associated with a successful effort to regulate deep body temperatures. When the initial core/environment differential was increased to 17° C degrees or more, however, puppies became hypothermic. At 35° C, a core/environment temperature differential of less than 2.5° C, hyperthermia was induced so rapidly and with such dramatic behavioural responses that observations were limited to intervals of 30 min. In all instances in which deviations of deep colonic temperatures occurred, normal body temperatures were resumed after incubation at 30° C.

Table 1 The behaviour of groups of beagle puppies (3 d old) segregated at selected environmental temperatures for 30 min

Effective environmental temperature (° C: mean $\pm$ s.e.)	No. of animals studied	Syndrome*		
		Exhibited	%	95% confidence intervals ¹⁰
35.2 $\pm$ 0.1	9	9	100	(77–100)
32.5 $\pm$ 0.1	13	5	39	(17–67)
30.1 $\pm$ 0.1	11	0	0	(0–25)
27.2 $\pm$ 0.1	14	3	21	(8–50)
25.1 $\pm$ 0.1	10	3	30	(12–62)
22.2 $\pm$ 0.2	10	7	70	(35–91)
19.9 $\pm$ 0.1	12	10	83	(60–97)
17.7 $\pm$ 0.1	8	6	75	(42–95)
14.8 $\pm$ 0.1	6	6	100	(60–100)

*Alertness, restlessness, hyperpnoea and vocalisation. Zero response indicated relaxation and somnolence.

These data indicate that the neonatal dog has a well delineated thermally neutral zone (TNZ) within which at rest it demonstrates a metabolic rate typical of a small mammal. At an ambient temperature which is within TNZ (30° C) it maintains a stable core temperature of the order of 37° C. This is similar to the mean core temperature which we have recorded in neonatal dogs of this age taken straight from the litter. These animals have a nycthemeral variation of less than 2° C. Already at this early age the dog is tachymetabolic and cenothermic¹¹.

It also displays distinctive and measurable physiological temperature regulation especially to cooling, but a relatively narrow yet clearly distinguishable tolerated ambient temperature range. This range lies approximately between 20° C and 30° C (Figs 1 and 2).

The behaviour of the neonatal dog at different ambient temperatures is especially revealing (Table 1). The animal appears to

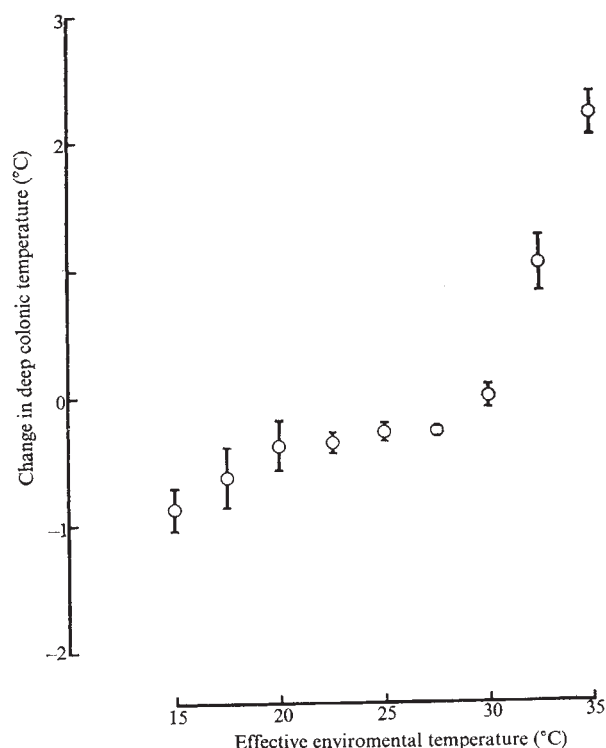


Fig. 2 Mean ($\pm$ s.e.) change in deep colonic temperatures of groups of neonatal beagle dogs (3 d old) segregated at selected environmental temperatures for 30 min.

Table 2 Deep colonic temperatures of beagle puppies 2 d old on exposure to different environmental temperatures

Environmental temperature (°C)	Deep colonic temperatures (°C: mean $\pm$ s.e.)	
	Time (min)	
	0	120
35.0	37.4 $\pm$ 0.1	39.5 $\pm$ 0.2*
30.0	36.8 $\pm$ 0.2	37.1 $\pm$ 0.1
25.0	37.5 $\pm$ 0.2	36.5 $\pm$ 0.4
20.0	37.2 $\pm$ 0.3	33.7 $\pm$ 0.4
8.0	36.9 $\pm$ 0.1	28.2 $\pm$ 0.5

*30 min interval

be largely indifferent to its surroundings when it is within TNZ. Within the range of tolerated ambient temperatures, in which by intrinsic means it is able to counteract the effects of thermal stress to sustain thermal balance, it demonstrates an awareness of its surroundings. At ambient temperatures which mark the upper and lower limits of temperature survival and which result in progressive deviations from the preferred core temperature of the neonate, onset of hypothermia or hyperthermia is not accepted voluntarily.

To consider fluctuations of core temperature in relation to ambient temperature alone and without reference to the presence of intrinsic thermoregulatory mechanisms and then go on to equate deviations of core temperature over selected ranges of ambient temperatures with a situation akin to poikilothermy may be misleading. We would cite the data in Table 2. At an effective environment temperature of 35° C, far from adopting this temperature as its core temperature, the core temperature of the neonatal dog rises dramatically. It is the presence rather than the efficacy of physiological temperature regulation which could well be the final arbiter when taxonomical difficulties are encountered.

Temperature regulation of the neonatal dog is admittedly very much more restricted compared with the adult, but this may be regarded as simply an expression of an immature phase in the development of what later becomes a highly efficient form of body temperature homeostasis in this species. We submit that our data go some way towards fulfilling the essential criteria by which one should judge a homeotherm and we conclude that the neonatal dog is an immature homeotherm, thus rejecting the hypothesis that it could be described as poikilothermic at this age.

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Cyclic AMP levels in *Phycomyces* during a response to light

ONE of the many metabolic functions attributed to adenosine 3':5'-cyclic monophosphate (cyclic AMP) is that of a mediator in the responses of living organisms to light stimulation. It has been associated with dopaminergic synaptic activities and also with the primary visual process in the retina. In the rod outer segments of the frog, *Rana pipiens*, cyclic AMP concentrations are diminished by illumination. Regulation is through the cyclic nucleotide phosphodiesterase¹. In addition, an adenylyl cyclase has been isolated from retina which is activated by the catecholamine dopamine; dopamine is thought to be an important neurotransmitter in the retina². The exact relationship between the initial transduction of the light signal and the attenuation in the levels of cyclic AMP is unknown. One reason for this is the complexity of the tissue and the difficulty of isolating clean fractions. I describe here a simpler system in which levels of cyclic AMP decrease in response to light stimulation much as in retina and where all the transduction occurs within a single cell.

The sporangiothore of the fungus *Phycomyces blakesleeanus* is a giant single cell—several cm long and 100 μ m in diameter—which responds in minutes to light, gravity, touch and the proximity of objects, the last being termed its autochemotropism (formerly avoidance). In a mature sporangiothore (stage IVb), the sensitive area is identical to the growing zone, a region 3 mm long, just below the apical sporangium, where essentially all elongation occurs³. In the case of the response to light, the nature of the behaviour has been worked out in detail using an automatic tracking system⁴, and several mutants have been isolated⁵. Figure 1 illustrates an example of a growth response to an increase in light intensity. The response is transient with a latency of about 3.5 min, maximal at 4-5 min and essentially complete after 12 min. The noise has been shown to be endogenous and to be associated with growth regulation after the reception of the light signal⁴. However, in spite of some initial efforts⁶, no chemical or morphological events have been shown to accompany or precede the observed acceleration of growth. I have investigated the levels of cyclic AMP in *Phycomyces* sporangiothores and found a sharp decrease well before the observed growth response.

P. blakesleeanus, NRRL1555, was grown on 4% potato dextrose agar supplemented with 0.1% yeast extract. Synchrony of sporangiothore development was achieved by a transfer from dark to light. The mature sporangiothores (3.5 $\pm$ 0.2 cm) were dark adapted for 40 min. At time zero they were exposed to 3 μ W cm⁻² continuous fluorescent light. At intervals 1 g of sporangiothores was plucked, quick frozen in liquid nitrogen and ground with a pestle and mortar. The fine powder was rehomogenised in perchloric acid and the supernatant liquid, after precipitation of proteins and KClO₄, was subjected to anionic exchange chromatography. A tracer of approximately 8,000 c.p.m. ³H-cyclic AMP was added at the homogenisation step to aid observation of the elution profile and assessment of final recovery. Recoveries varied from 75 to 95%. However, only the middle 40% of the cyclic AMP peak eluted from the column was pooled, concentrated and assayed. Final results were corrected for recovery and subfractionation. I used the assay of Brown *et al.*⁷ which is a modification of the competitive binding protein assay of Gilman⁸. The amounts of cyclic AMP found in dark adapted sporangiothores varied from 0.1 to 0.3 nmol g⁻¹ of wet weight. Figure 2 illustrates the normalised

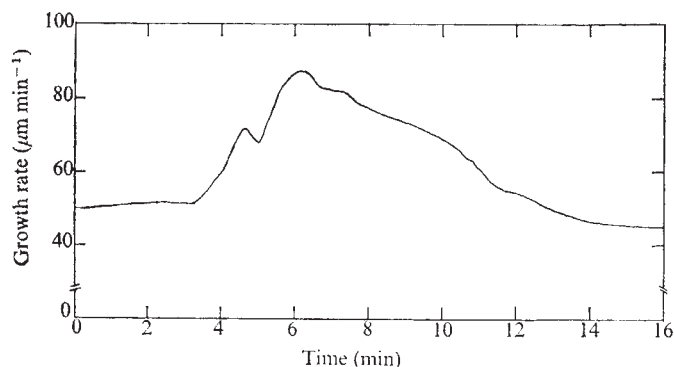


Fig. 1 A typical growth response of *Phycomyces blakesleeanus* to an increase of light intensity at 24°C. Conditions are comparable with those used to assay cyclic AMP. The adapting light intensity was in the normal range, less than about 20 $\mu\text{W cm}^{-2}$ and the final light intensity was sufficient to saturate the response system. The step-up was imposed at time zero. The latency was 3.5 min.

results of assays for cyclic AMP during a response to continuous light.

Cyclic AMP levels decreased precipitously to about 50% of the initial dark value within 1 min of light stimulation, well before the appearance of a growth response. Indeed the level had returned to approximately the preillumination value at about the time that growth was first observed. This is the first direct *in vivo* demonstration of a change in cyclic AMP level in a responsive system after light stimulation. The sharp decrease in cyclic AMP suggests that the light receptor for *Phycomyces* is a nucleotide cyclase or possibly a cyclic mononucleotide phosphodiesterase, or may be closely associated with one of these enzymes such that light inhibits adenylyl cyclase or activates phosphodiesterase. Thus the primary transduction event may be the attenuation of cyclic AMP concentration. Goridis and Virmaux⁹ hypothesised that attenuation of phosphodiesterase is the general mechanism for the function of all sensory receptors.

Because the slight decrease in cyclic AMP during the transient growth response was unexpected, the investigation was extended beyond the response (Fig. 2). I found that levels of cyclic AMP had stabilised after the response; the levels in

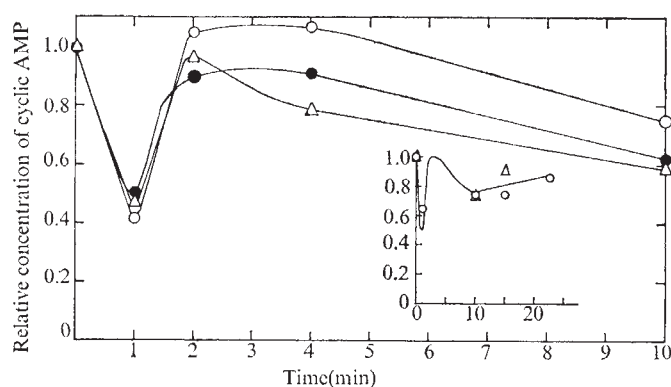


Fig. 2 The normalised level of cyclic AMP in stage IVb sporangiophore as a function of the time after a light step-up at time zero. The curves refer to three separate runs, each point being the mean average of five to eight determinations. The absolute amounts of cyclic AMP initially present were: $\circ$, 150; $\bullet$, 302, $\triangle$, 113 pmol g^{-1} wet weight. Standard deviations of the mean ranged from 10 to 15%. Temperature was 24°C. The amount of protein in these sporangiophores was 5.0 mg g^{-1} wet weight as measured by the method of Lowry. Details of the procedure are given in the text. The inset shows levels of cyclic AMP in stage IVb sporangiophores extended to 25 min after the increase. Other details are as in the rest of the figure. The absolute amounts of cyclic AMP initially present were: $\circ$, 273 and $\triangle$, 282 pmol g^{-1} wet weight.

continuous light were perhaps slightly lower in these experiments than during continuous darkness.

Because levels of possible interfering substances have not been investigated thoroughly in fungal material, I was careful to eliminate factors other than cyclic AMP that might affect my measurements. The validity of the reported estimates is supported by (a) the careful ion-exchange column isolation; (b) the fact that when isolate was subjected to prolonged treatment with rat brain phosphodiesterase¹⁰, boiled and reassayed, no cyclic AMP was found, while in a parallel experiment without this treatment the usual amount was found, and (c) several dilutions of cyclic AMP isolate yielded identical initial concentrations.

Preliminary investigations indicate a very high adenylyl cyclase activity consistent with high levels of product. Cyto-

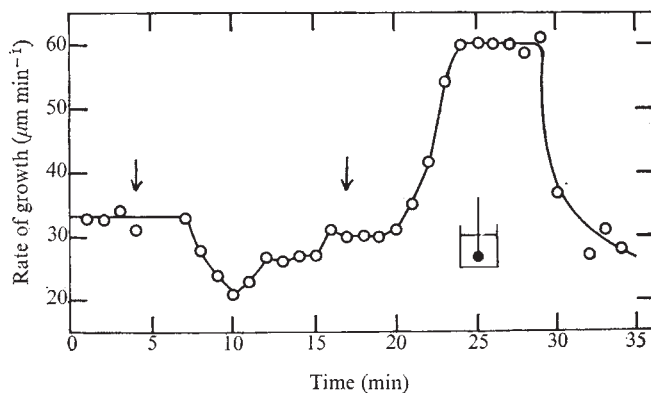


Fig. 3 The response to the addition of dibutyl cyclic AMP (indicated by left hand arrow) to a growing sporangiophore. The sporangiophore was initially suspended in fluorocarbon so that the growing zone was submerged as illustrated. The nucleotide was added with careful stirring so as to make a final concentration of 1 mg ml^{-1} in 1.4 ml. The reproducibility of the response was quite good—in five separate measurements the latency was 3.5 min and the mean decrease at the minimum was $32\% \pm 3\%$ (s.d.). The growth response to a bilateral blue light stimulation (at right hand arrow) was normal in the presence of dibutyl cyclic AMP; (0.25% Triton X-100 was present to solubilise the nucleotide. Neither this nor stirring alone caused any discernable changes in growth rate.)

plasm from *Phycomyces* sporangiophores grown and assayed in continuous light has a V_{max} of 1.3 nmol of cyclic AMP produced per min per mg of protein and a high affinity for ATP ($K_m = 0.5$ mM) as assayed by a slight modification of the method of Bär and Hechter¹¹.

Adenylyl cyclase has been localised cytochemically using the specific inhibitor adenylyl imidodiphosphate by a method developed for islets of Langerhans¹². Cyclase is abundant in the growing zone of the sporangiophore and is not present in the nongrowing zone areas. The plasmalemma, mitochondria and cytosol all have significant activity (my observations with C. J. M. Farnham).

One might expect then that exposure to cyclic AMP would induce a transient negative growth response. The sporangiophore of *Phycomyces* grows poorly under water but well in the fluorocarbon perfluorotributylamine. In such a system, chemicals can be added and the response of the sporangiophore observed. The introduction of dibutyl cyclic AMP at a final concentration of 1 mg ml^{-1} consistently leads to a transient 30% decrease in the rate of growth (Fig. 3) indistinguishable from the response to the dimming of light. Results obtained with the phosphodiesterase inhibitor theophylline are similar. This again is consonant with the hypothesis that in *Phycomyces*, modulation of cyclic AMP concentration is an important link between the reception of light and the observed transient changes in growth rate.

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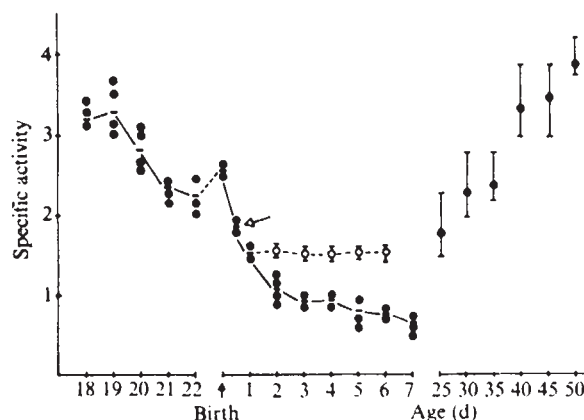


Fig. 1 Enzyme activity of alcohol dehydrogenase (mU mg^{-1} protein) in the testis of foetal and postnatal control rats and in chronically hydrocortisone treated rats. Hydrocortisone acetate was purchased as Hydrocortison in a watery suspension (25 mg ml^{-1}) from Hoechst AG, Frankfurt, West Germany. ●, Controls; arrow, controls 8 h after birth (each circle represents one experiment); ○ animals treated daily with 1.25 mg hydrocortisone subcutaneously from the first day after birth till to day 6 of life. Preparation of the testes was performed 7 h after the last injection. In hydrocortisone-treated rats and in control rats between days 25 and 50 of age at least three experiments were performed. The main values and deviations are included.

Glucocorticoids and hCG sensitivity of rat testicular Leydig cell

THERE is unequivocal evidence that the foetal Leydig cell in the rat is under the control of gonadotrophins¹ whereas the postnatal Leydig cell is insensitive to hypophyseal luteinising hormone (LH) (ref. 2) as well as to exogenous human chorionic gonadotrophin (hCG) (refs 3 and 4). We have shown recently that the hCG insensitivity of the Leydig cell during the first 2 weeks after birth is not due to the lack of the specific hCG receptor nor of an enzyme adenyl cyclase, both of which are prerequisites of hCG action^{3,4}. The receptor is a constitutive protein which is already present in the foetal Leydig cell⁵.

It seems obvious that the apparent loss of hCG sensitivity of the Leydig cell after birth must be causally connected with the separation of the foetus from the mother. It seems likely that the cells are deprived of a stimulus for growth and activity. This stimulus might be another hormone which is present in the rat foetus, absent after birth and simultaneously restored with the onset of hCG sensitivity 2 weeks later. The glucocorticoids of the adrenal cortex fulfil such a requirement. The plasma corticosterone level is high before and shortly after birth⁶, decreases to a very low level in the 12 d old rat⁷ and increases again afterwards⁸. Therefore we have tested whether hCG sensitivity during the insensitive period is inducible by hydrocortisone and adrenocorticotrophic hormone (ACTH) respectively. Alcohol dehydrogenase (ADH; E.C. 1.1.1.1), an enzyme specifically located in the Leydig cell was used as a marker for Leydig cell function.

Rats of strain SIV-50 were used throughout the experiments. For the study of prenatal animals at a definite developmental stage, mating was determined by examining the presence of copulation plugs in the female rats (day of vaginal plug = day 1 of pregnancy). At the respective experimental days of pregnancy the animals were killed, exsanguinated and the testes of the fetuses were removed. Varying numbers of animals were used in each experiment dependent on the age of animals: 20-40 fetuses, 15-20 animals between birth and day 8, 10 animals between days

9 and 13, five animals between days 14 and 16 and one animal between days 25 and 50. Each experiment was done at least in triplicate, if not otherwise stated. The procedures used for the preparation of the animals and for ADH activity measurements were similar to those described earlier⁹.

As shown in Fig. 1 in the day 19 foetal rat testis ADH activity is as high as in the 45 d old rat when spermatogenesis is fully established. During late foetal development testicular ADH activity decreases, but the most dramatic decrease occurs during the first 2 d after birth; the lowest activity is found at 11 d of age⁴. Daily injections of 1.25 mg hydrocortisone from day 1 after birth onwards till day 6 of life prevent the decrease in ADH activity. Furthermore, by daily injections of 80 IU hCG in addition to hydrocortisone even the foetal enzyme activity level is restored (not shown).

We have reported recently that the postnatal Leydig cell, in spite of an intact apparatus for the response to hCG, is insensitive to the hormone during the first 2 weeks after birth^{3,4}. Figure 2 shows that the cell becomes sensitive to gonadotrophins after hydrocortisone or ACTH treatment. After a single injection of hydrocortisone and hCG simultaneously, on each experimental day, on testicular ADH activity increases to a greater extent than after the injection of hydrocortisone alone.

Figure 3 shows that a single simultaneous injection of 2.5 mg of hydrocortisone and 80 IU hCG into rats at day 6 and day 8 after birth results in a maximum increase of testicular ADH activity 31 h later. Afterwards the activity decreases, and reaches nearby control levels 80-100 h after the injection. Since exogenous hCG cannot stimulate ADH activity during this developmental period the decrease must be due to hydrocortisone degradation during the experimental time interval. These results indicate that an adequate glucocorticoid level has to be maintained in order to guarantee hCG responsiveness of the Leydig cell. As a nearly adult plasma corticosterone level is achieved in the 21-d-old male rat⁶, hydrocortisone injection into rats older than 21 d should be ineffective. Accordingly, testicular ADH activity in hydrocortisone-treated 26-d-old (5.0 mg per animal) and adult animals (12.5 mg per animal) corresponds to control animals 24, 31, 58, 72 and 96 h after the injection (not shown).

From our results we infer that the insensitivity of the postnatal Leydig cell to gonadotrophins is due to the lack of glucocorticoids during this developmental period. The foetal adrenal cortex begins to secrete glucocorticoids on gestational day 16 or 17 (ref. 9) and could therefore provide the natural stimulus for the LH sensitivity of the foetal Leydig cell. The maximal concentration of corticosterone in the plasma and adrenal glands occurs between days 18 and 19 and then decreases till birth⁶. The hypothalamic control of the pituitary-adrenal system begins to operate between days 18 and 22 of gestation¹⁰. After birth, however, the pituitary-adrenal axis becomes dormant and is re-established approximately 2 weeks later; the normal control of ACTH release emerges from day 11 to maturity¹¹. Our ADH activity measurements in foetal and postnatal testicular tissue, as well as after hydrocortisone or ACTH treatment, are in line with these findings.

Obviously, the adrenal glucocorticoids are necessary for

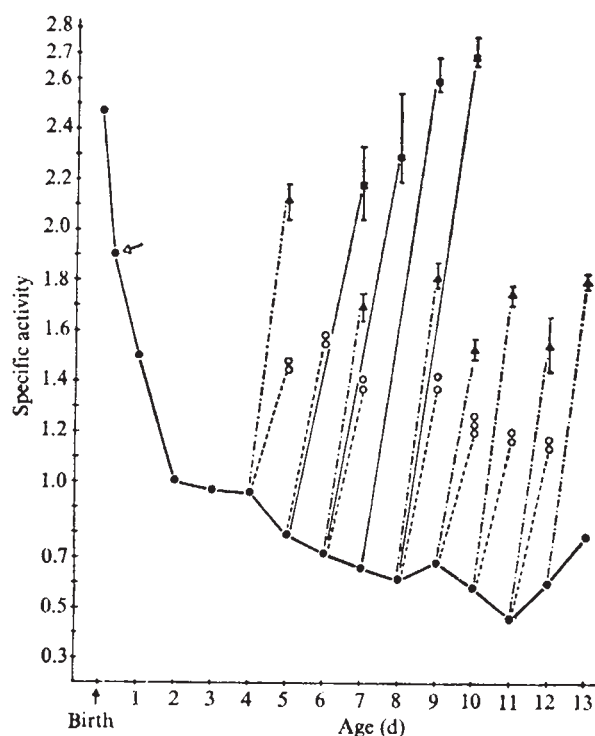


Fig. 2 Enzyme activity of alcohol dehydrogenase (mU mg^{-1} protein) in the testis of rats between birth and the day 13 of age in controls and treated animals. $\bullet$, Controls; $\rightarrow$, controls 8 h after birth (for further details see Engel and Frowein⁴); $\circ$, animals treated with 2.5 mg hydrocortisone subcutaneously and prepared 31 h later. Each circle represents one experiment. $\blacktriangle$, Animals were injected simultaneously with 2.5 mg hydrocortisone intraperitoneally and 80 IU hCG, subcutaneously, dissolved in 0.1 ml of a 0.9% NaCl solution (preparation of the testes was done 31 h after the injections). $\blacksquare$, After two doses of 1 IU ACTH (dissolved in 0.1 ml of a 0.9% NaCl solution) subcutaneously at a 24 h interval. The testes were removed 7 h after the last injection. Mean values and deviations resulting from at least three experiments with hCG+hydrocortisone ($\blacktriangle$) and ACTH ($\blacksquare$) are demonstrated. HCG was supplied as Primogon (crude hCG preparation, only hCG activity; specific activity of $3,000 \text{ IU mg}^{-1}$ protein) from Schering AG, Berlin, West Germany. Two ACTH preparations were used: Depot-Acethropan (highly purified preparation from animal hypophysis; salt-like bound to polyphosphoric acid; exclusive ACTH activity; specific activity of 200 IU mg^{-1} protein) from Hoechst AG, Frankfurt, Western Germany and Depot-Synacthen (synthetic ACTH preparation; β^{1-24} corticotrophin hexa-acetate; the first 24 amino acids are identical to the genuine human ACTH) from CIBA-Geigy AG, Wehr, Western Germany. The results obtained with both ACTH preparations were identical.

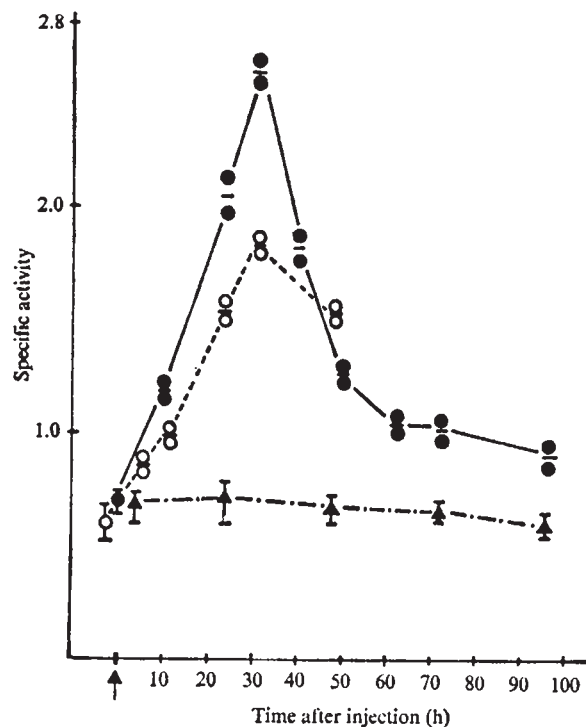


Fig. 3 Enzyme activity of alcohol dehydrogenase ($\text{mU} \times \text{mg}^{-1}$ protein) in the testis of rats at day 6 ($\bullet$) and day 8 ($\circ$) of life, prepared between 5 and 98 h after a single simultaneous injection ($\rightarrow$) of 2.5 mg hydrocortisone (intraperitoneally) and 80 IU hCG (dissolved in 0.1 ml 0.9% NaCl solution) subcutaneously. Each circle represents one experiment. Also ADH activities in rats between days 6 and 10 of age, daily treated with hCG, subcutaneously, from birth onwards ($\blacktriangle$) are included in the figure; for further detail see Engel and Frowein⁴.

the initiation of the sexual maturation process in the male rat. Recently Gorski and Lawton¹² and Ramaley¹³ have provided evidence that the adrenals play a definite role in determining the time of onset of puberty in the female rat. In immature or adult male rats cortisone has been reported to stimulate testis weight and to effect testicular morphology (for review see Gomes¹⁴). Zondek and Zondek¹⁵ found a positive correlation between hypoplasia of the adrenal cortex and the number of Leydig cells in anencephalic human fetuses. Brook *et al.*¹⁶ reported a 15.9-yr-old boy with congenital adrenal hypoplasia exhibiting no signs of puberty. Furthermore, in men with Addison's disease Leydig cells seem to be sensitive to hCG only to a very small extent (E. Nieschlag, unpublished). ACTH injections into prepubertal children result in a significant increase of plasma testosterone and dehydroepiandrosterone concentration¹⁷.

The question arises by which mechanism glucocorticoids may provoke hCG sensitivity of the Leydig cell. We have shown recently that hydrocortisone-1-2-³H can bind to a cytoplasmic receptor in rat testicular tissue (W.E., and J.F., unpublished). In contrast to liver, however, the hydrocortisone receptor complex is not translocated to the nucleus (J.F. and W.E., unpublished). Since hydrocortisone has a similar effect on ADH activity in the hCG insensitive testis as theophylline^{3,4}, we assume that glucocorticoids act by alteration of cyclic 3', 5'-monophosphate phosphodiesterase activity in the Leydig cell. We already have experimental evidence supporting this view (W.E., and J.F., unpublished). A similar effect of the glucocorticoids has been described in various other tissues¹⁸⁻²⁰.

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Indomethacin potentiates PGE₁ stimulated cyclic AMP accumulation in human synoviocytes

PROSTAGLANDINS have both inhibitory and stimulatory effects on inflammatory and immune processes¹. The capacity of the nonsteroidal anti-inflammatory agents, aspirin and indomethacin (IM), to inhibit the synthesis of certain prostaglandins emphasises the potential importance of this hormone system in the pathogenesis and treatment of inflammatory arthritis². Since the prostaglandins inhibit certain cellular functions involved in the production of some forms of arthritis³, suppress the inflammatory response of adjuvant arthritis⁴⁻⁷ and yet induce an acute inflammatory response when injected into the dog stifle joint⁸, there remain certain paradoxes with regard to the role of prostaglandins in inflammatory joint diseases.

We have used human synoviocytes in culture to investigate directly the effects of IM on these cells in the presence and absence of prostaglandins. We report here results which demonstrate a significant increase in the intracellular concentration of cyclic AMP when human synoviocytes in culture are treated with PGE₁. Our results also show this hormone-mediated effect to be potentiated by IM, an inhibitor of human synoviocyte cyclic adenosine 3',5'-nucleotide phosphodiesterase (PDE) activity.

Human synovial lining (SLC) or tendon sheath lining (TLC) cells were dissected from underlying tissue at surgery by using a dissecting microscope, cut into small pieces and planted in growth media. When sufficient outgrowth was observed, cultures were trypsinised and subcultured for experimental use.

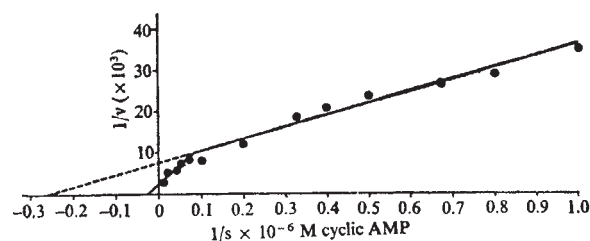


Fig. 1 Lineweaver-Burk plot of synoviocyte cyclic adenosine 3', 5'-monophosphate phosphodiesterase activity. Each point represents the average of two determinations, and the slope and intercept calculations are by linear least square analysis. The maximal velocities for the low and high K_m plot are 135 and 435 pmol cyclic AMP hydrolysed per 30 min, respectively.

Subcultured cells showed ultrastructural characteristics similar to those published for human type A and B synoviocytes in tissue culture^{9,10} and produced significant amounts of hyaluronate using a qualitative mucin test¹¹. Cells were routinely split 1 to 2 h after the first subculture had reached confluency; thus, the passage number roughly corresponds to the number of cell doublings after the first subculture. Cells were used between the fourth and twelfth passage and were mycoplasma negative. Protein was measured by the Lowry method¹², and the Thompson and Appleman method¹³ was used to determine PDE activity. ³H-cyclic AMP (28 Ci mmol⁻¹, Schwarz-Mann) was purified by thin layer chromatography¹³.

The control values for cyclic AMP in the experiments presented (Table 1) are somewhat higher than have been determined for other nonestablished cell lines. When human synoviocytes were assayed without a 1 h preincubation in serum-free media, however, we found the cyclic AMP concentration to be 18.9 ± 2.66 pmol per mg protein which is similar to the concentrations found by others in quiescent fibroblasts¹⁴. The absence of serum for 1 h results in a two to threefold increase in intracellular cyclic AMP concentrations which agrees with the results of Froehlich and Rachmeler¹⁵ who found an inverse relationship between intracellular cyclic AMP and media serum concentrations for human diploid fibroblasts. Ethanol at concentrations up to 0.53% had no significant effect on the intracellular cyclic AMP concentrations.

IM used alone at concentrations of 10^{-4} and 10^{-5} M had little effect on intracellular cyclic AMP concentration (Table 1, experiments 1 and 2). IM at 10^{-6} M resulted in a modest decrease in cyclic AMP concentration (Table 1, experiment 1) but, the addition of PGE₁ plus IM (10^{-4} to 10^{-6} M) routinely caused a potentiation of the PGE₁ response in both TLC and SLC cell lines. IM added to the cells in increasing concentrations resulted in a concentration-dependent increase in intracellular cyclic AMP over the PGE₁-stimulated levels (Table 1, column 4). The potentiation of the PGE₁ effect on intact cells occurs at concentrations of IM (10^{-5} M) known to give effective anti-inflammatory responses *in vivo*. Our data (Table 1, experiment 1) demonstrating a modest decrease in intracellular cyclic AMP concentrations with very low concentrations of IM alone (10^{-6} M) suggest a mechanism involving prostaglandin synthetase (PS) inhibition. At high IM concentrations, however, both PS and PDE activities might be inhibited resulting in little change in cyclic AMP. The PGE₁-dependent effect of IM may be the result of an effect of IM on either cyclic AMP or PGE₁ metabolism or an effect of PGE₁ on IM metabolism. The observations that theophylline, an inhibitor of PDE activity, also potentiated the PGE₁-responsiveness of human synoviocytes (Table 1, experiment 4) and that IM is an inhibitor of epiphyseal cartilage PDE activity¹⁶ led us to examine the effect of IM on synoviocyte PDE activity.

TLC homogenates demonstrated linear PDE activity up to protein concentrations of $185 \mu\text{g ml}^{-1}$ using 1×10^{-6} and 1×10^{-4} M cyclic AMP, respectively. Kinetic studies (Fig. 1),

using conditions in which less than 20% of the substrate was converted to product, provided data that gave nonlinear Lineweaver-Burk plots with two apparent Michaelis constants for cyclic AMP (4.0×10^{-5} M and 3.9×10^{-6} M). Indomethacin at a final concentration of 10^{-3} M resulted in 89% inhibition of cyclic AMP-PDE activity with 1×10^{-6} M cyclic AMP (Table 2) and only 10.8% inhibition with 1×10^{-4} M cyclic AMP as substrate. The concentration of IM causing 50% PDE inhibition was 1.35×10^{-4} M. Significant inhibition of PDE activity with low and not with high substrate concentrations demonstrates a specificity for the low K_m enzyme which is presumed to represent the enzyme species most important for the physiological control of intracellular cyclic AMP concentrations. The concentrations of IM to obtain 50% inhibition of the PDE activity do not reflect therapeutic concentrations of the drug. Since the lysosomal system, however, accounts for the cellular uptake of indomethacin²¹, intracellular concentrations may be higher than the serum concentrations of IM stated as effective for therapeutic action. In addition, the loss of cell membrane integrity which results in altered receptors, transport, and concentrating mechanisms in broken cell preparations makes this assay a less competent system for studying physiological PDE activity.

Table 1 The effect of PGE₁ and indomethacin (IM) on intracellular cyclic AMP concentration

Cell type		Cyclic AMP pmol per mg cell protein	Increment in cyclic AMP concentration above PGE ₁ - stimulated level (%)
TLC (Experiment 1)	Control (0.2% ethanol)	103.0 ± 18.2	
	IM (10^{-4} M)	84.0 ± 11.0	
	IM (10^{-5} M)	87.3 ± 21.4	
	IM (10^{-6} M)	68.4 ± 22.4	
SLC (Experiment 2)	Control (0.2% ethanol)	56.7 ± 19.4	
	PGE ₁	468.6 ± 45.4	
	IM (10^{-4} M)	92.7 ± 17.8	
	PGE ₁ +IM (10^{-4} M)	869.1 ± 107.1	85.47
TLC (Experiment 3)	Control (0.2% ethanol)	74.3 ± 7.0	
	PGE ₁	1,423.7 ± 40.5	
	PGE ₁ +IM (10^{-5} M)	2,009.8 ± 57.2	41.18
	PGE ₁ +IM (10^{-6} M)	1,690.1 ± 98.6	18.76
SLC (Experiment 4)	Control (0.53% ethanol)	60.5 ± 5.2	
	PGE ₁	156.2 ± 39.3	
	PGE ₁ +7-oxa-13- prostynoic acid (50 µg ml ⁻¹)	79.5 ± 24.4	
	PGE ₁ +theophyl- line (10^{-3} M)	848.6 ± 175.2	
	PGF _{1α}	38.8 ± 8.3	

Human synoviocytes isolated from either tendon (TLC) or synovial (SLC) linings were planted in 60×15 mm plastic culture dishes and grown to confluency using BME (Gibco) supplemented with 20% foetal calf serum, 2 mM L-glutamine, 100 units ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. Following a 60 min preincubation at 37° C in serum-free media, the cells were treated with either indomethacin at a final concentration between 10^{-4} and 10^{-6} M, theophylline at 10^{-3} M or an equal volume of 100% ethanol for 15 min. Subsequently the cells were treated for another 15 min with either PGE₁ (3 µg ml⁻¹), PGF_{1α} (3 µg ml⁻¹) or an equal volume of 100% ethanol. 7-oxa-13-prostynoic acid (50 µg ml⁻¹) was added in appropriate incubations together with PGE₁. All monolayers were processed by the method of Manganiello and Vaughan¹⁶. Cyclic AMP was assayed by the protein-binding method of Gilman¹⁷. All test compounds were solubilised in small volumes of 100% ethanol except for theophylline which was added in BME. All incubations were done on duplicate plates and cyclic AMP assayed at two dilutions. Appropriate control samples were assayed for cyclic AMP after the addition of purified beef heart PDE to determine the amount of nonspecific binding¹⁸. All values shown have been corrected for this nonspecific binding. Each cyclic AMP concentration given represents the mean ± s.d. of four determinations. Final concentrations in the incubation are given for each treatment.

These results and others showing that IM causes an increment in cyclic AMP in the presence of PGE₁ receptor-site saturation (D.S.N. and C.P.C. unpublished) suggest that the IM potentiation of PGE₁ stimulation of cyclic AMP is most likely the result of PDE inhibition.

In addition to the IM effect on cyclic AMP, our experiments (Table 1) demonstrate a direct effect of PGE₁ on human synoviocytes. PGE₁ increased the intracellular cyclic AMP concentration in human synoviocytes derived from both tendon sheath and synovial membrane.

The specificity of the PGE₁ response is supported by the fact that PGF_{1α} did not cause an increase in intracellular synovio-cyte cyclic AMP concentration, and that 7-oxa-13-prostynoic acid, a competitive inhibitor of prostaglandin binding in some systems²², depressed PGE₁ responsiveness (Table 1, experiment 4).

Our results show that IM potentiates the effect of PGE₁ on intracellular cyclic AMP concentration by inhibiting synovio-cyte PDE activity. Since prostaglandins are found in inflammatory exudates¹, the PGE₁ effect amplified by IM may represent a new component of the anti-inflammatory effect of this drug. We have also shown a PGE₁-mediated stimulation of human synoviocytes. The effects of PGE₁ and IM on intracellular cyclic AMP concentration suggests that, as has been shown for other tissues²³, the inflammatory and immune responses of human synoviocytes may be regulated by this mechanism.

Table 2 Inhibition of human synovio-cyte cyclic nucleotide phosphodiesterase activity by indomethacin.

Indomethacin concentration (M)	PDE specific activity (units per mg protein) 10^{-6} M cyclic AMP as substrate	Inhibition (%)
Control	543	—
10^{-3}	61	89
10^{-4}	389	28
10^{-5}	516	5
10^{-6}	525	3

Monolayers were washed five times in homogenising buffer (0.04 M Tris-HCl, pH 8.0 containing 10.9% sucrose) at 4° C. All homogenates were prepared by the method of D'Armiento *et al.*²⁰, and 25×10^6 cells per 7.5 ml of homogenising buffer resulted in a protein concentration of 0.775 mg ml⁻¹. One unit of PDE activity was defined as the amount of enzyme required to hydrolyse 1 pmol cyclic AMP per 30 min at 30° C. Indomethacin was solubilised in DMSO, and control and experimental incubations contained in final concentration 1.25% DMSO. Appropriate control incubations established that the observed effects of IM on PDE activity were not the result of 5'-nucleotidase inhibition. All assays were run in triplicate.

These investigations establish a role for prostaglandins and cyclic AMP in the biological function of human synoviocytes and propose a new mechanism for the anti-inflammatory activity of indomethacin.

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effective when added to the mucosal, serosal, or both solutions, but in the experiments reported here it was added symmetrically to both sides of the preparation.

Addition of TAP invariably increases the membrane resistance (R_m) and decreases the P_{Na}/P_{Cl} ratio. Both effects are totally due to a specific decrease in G_{Na} . Let us define the fractional inhibition (α) of the partial conductance of an ion X as $\alpha_X \equiv 1 - [G_X(TAP)/G_X(\text{control})]$, where $G_X(\text{control})$ and $G_X(TAP)$ are the conductances of the ion species X in the control period and in the presence of TAP respectively. Each point of Fig. 1b represents an experimental α_{Na} determination in an individual frog gallbladder at a given TAP concentration. The figure shows that as the TAP concentration increases α_{Na} approaches 1, that is, G_{Na} is reduced. At 19 mM 92% of G_{Na} is inhibited. The dependence of α_{Na} on TAP concentration suggests a saturation kinetics. Therefore, the experimental values were fitted by the equation:

$$\alpha_{Na} = C [\alpha_{Na}(\text{max})] / [K_m + C] \quad (1)$$

where C represents the concentration of TAP, and $\alpha_{Na}(\text{max})$ and K_m are two constant parameters. The curve drawn through the experimental α_{Na} values of Fig. 1b is based on the least mean squares fit of the experimental values to equation 1. The fitted parameters and their standard deviation are $K_m = 2.6 \pm 0.1$ mM, and $\alpha_{Na}(\text{max}) = 1.0 \pm 0.0$. This indicates that 100% of G_{Na} can be inhibited by TAP at sufficiently high concentrations, and that the inhibitory process can be described by a single constant.

The situation for G_{Cl} , illustrated in Fig. 1a, is very different: at all TAP concentrations tested $\alpha_{Cl} \approx 0$, meaning that TAP does not affect G_{Cl} . This indicates that Na^+ and Cl^- migrate through separate pathways, and is in agreement with previous evidence⁴ suggesting that the Cl^- pathway is a relatively small ($G_{Cl}/G_{Na} \approx 0.2$ in untreated frog gallbladder) parallel leakage or free solution shunt.

In five other leaky epithelia¹ (six rabbit gallbladders, two *Necturus* gallbladders, two rabbit small intestines, two bullfrog small intestines, and two bullfrog choroid plexuses) TAP had qualitatively the same effect as in frog gallbladder. In all cases TAP increased R_m and decreased P_{Na}/P_{Cl} , and the effect could be completely attributed to an inhibition of G_{Na} .

Blockage of cation permeability across the tight junctions of gallbladder and other leaky epithelia

THE main route of passive ion permeability in gallbladder and other 'leaky' epithelia is through the so-called 'tight' junctions between cells, not through the cells themselves¹. I report here that the protonated form of the organic base 2,4,6-triaminopyrimidine (TAP), reversibly and specifically blocks cation permeation across this tight junction route in frog gallbladder and several other leaky epithelia. Since in these epithelia the tight junction permeability is mainly (or even completely) cationic, in principle on addition of TAP a low resistance, leaky epithelium can become a high resistance epithelium.

Transepithelial electrical potential differences (p.d.) and conductances (G) were measured across flat sheets of bullfrog gallbladder mounted between two well-stirred chambers at 23° C. The experimental procedure, and methods for obtaining ionic permeability (P_{ii}) and conductance (G_i) values (correcting G_{Na} for a small leakage current) were described previously². The rate of fluid transport was measured in 'sac' preparations of bullfrog gallbladder by the gravimetric procedure described in detail by Diamond³. Since 2,4,6-triaminopyrimidine ($pK_a = 6.72$) is only active in the protonated form (manuscript in preparation), all the experiments were done at pH 6.0, and only concentrations of the protonated form are reported. TAP was

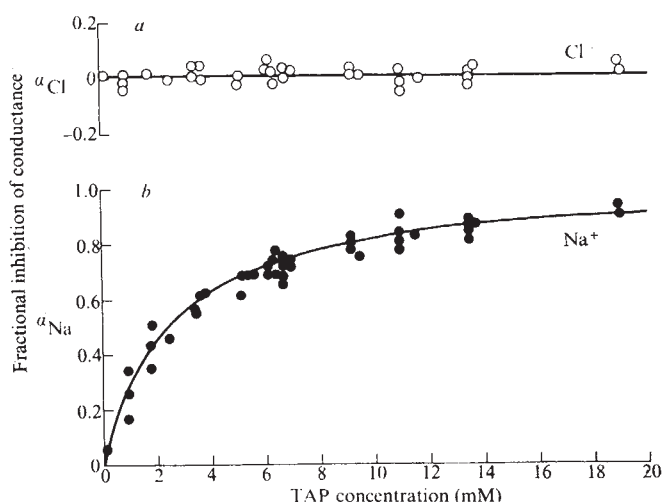


Fig. 1 Effect of TAP on G_{Na} and G_{Cl} . The fractional inhibition of G_{Na} ($\bullet$, α_{Na} , below) and G_{Cl} ($\circ$, α_{Cl} , above) are plotted against TAP concentration. Each point corresponds to one experimental value. Notice that G_{Na} is strongly inhibited (α_{Na} increases) as TAP concentration increases, whereas G_{Cl} is unaffected. The line drawn through the α_{Na} values is the least mean squares fit to equation 1 yielding $K_m = 2.6$ mM, $\alpha_{Na}(\text{max}) = 1.0$. The line through the α_{Cl} values is the line of identity $\alpha_{Cl} = 0$.

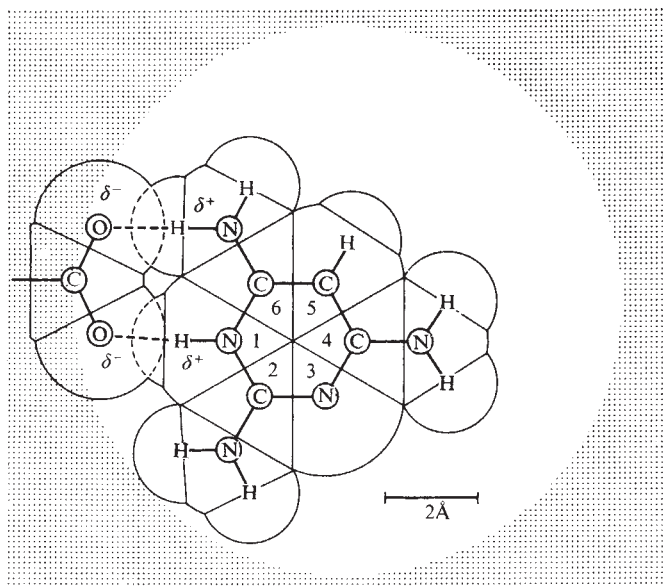


Fig. 2 Scale drawing of a molecule of TAP associated by two hydrogen bonds to a carboxylic group. The outline gives the van der Waal's surface, the heavy lines inside represent the internal covalent bonds, the broken lines represent hydrogen bonds. The van der Waal atomic radii, bond lengths and angles are taken from Corey-Pauling-Koltun models. Notice the good fit of the two hydrogen bonds. If a $-\text{PO}_4\text{H}^-$ group is used instead of a $-\text{COO}^-$ group, the fit of the hydrogen bonds is equally good. The shadow limit represents a ring of radius 6 Å, an equivalent radius attributed to bullfrog gallbladder tight junction cation channels^{2,4}.

The gallbladder actively transports an isotonic solution from the mucosal side. The rate of this fluid transport measures (and is caused by) active transport of Na^+ from the mucosal to the serosal solutions³. In the absence of NaHCO_3 in the solutions the rate (average $\pm$ s.e.) of the fluid transport in six frog gallbladders was $3.9 \pm 0.6 \mu\text{l h}^{-1} \text{cm}^{-2}$, and after addition of 10 mM TAP, $3.8 \pm 0.8 \mu\text{l h}^{-1} \text{cm}^{-2}$. When 7 mM NaHCO_3 (which stimulates active transport³) was included in the bathing solutions, the rate of transport increased to 9.8 ± 1.5 ($n = 3$) $\mu\text{l h}^{-1} \text{cm}^{-2}$ in the control, and to 9.9 ± 1.9 ($n = 3$) $\mu\text{l h}^{-1} \text{cm}^{-2}$ in the presence of 10 mM TAP. Therefore TAP at a concentration which blocks 80% of the transepithelial Na^+ conductance does not seem to interfere with active Na^+ transport in frog gallbladder nor affect HCO_3^- stimulation of transport.

A similar conclusion can be reached from a completely different observation: in 24 frog gallbladders TAP (5–11 mM) increased R_m and the spontaneous transepithelial p.d. (V_m) from $129 \pm 7 \Omega \text{cm}^2$ and $2.2 \pm 0.2 \text{ mV}$ (serosa positive), to $257 \pm 12 \Omega \text{cm}^2$ and $4.8 \pm 0.4 \text{ mV}$ respectively. But the ratio V_m/R_m (analogous to an open circuit current and hence a measure of active Na^+ transport) did not change significantly ($17.1 \pm 1.4 \mu\text{A cm}^{-2}$ for the control, against $18.3 \pm 1.3 \mu\text{A cm}^{-2}$ after TAP). These results add to the growing evidence in support of an electrogenic Na^+ transport rather than the original suggestion⁵ of an electrically neutral mechanism as responsible for active Na^+ transport in gallbladder (see ref. 4 for further discussion.)

Therefore, I conclude that in frog gallbladder TAP acts specifically in the cation-selective ion channels of the tight junction responsible for the passive Na^+ permeability^{1,4} without affecting either the small Cl^- leakage current or the active Na^+ transport.

From studies on the cation permeability and selectivity in frog gallbladder^{2,4} these channels are thought to be hydrated and relatively large (the estimated radius is 6–8 Å). Their Na^+ conductance is controlled by acidic groups whose apparent pK_a is 4.4. The cation ligands in the channel (probably the same acidic groups) behave as strong hydrogen bond proton acceptor

sites which distinguish more strongly among cationic proton donors than does water⁶.

The protonation of TAP occurs at one of the ring nitrogen atoms (see Fig. 2), but the net charge is delocalised by resonance, and shared with the 2,4 and 6 amino groups⁷. Therefore, TAP should be a strong H bond proton donor. The H bond association illustrated in Fig. 2 between a molecule of TAP with a $-\text{COO}^-$ group (or a $-\text{PO}_4\text{H}^-$ group, although it is not shown in the figure) could be particularly strong, because the atoms involved in the H bonds have high partial ionic character and form appropriate angles between the internuclear lines (Fig. 2). Actually, ion pair formation between phosphate ions and 2,4-diaminopyrimidine in relatively dilute aqueous solutions has been reported and attributed to H bond associations such as those illustrated in Fig. 2 (ref. 7). Indeed, these characteristics of TAP as a strongly H bonding proton donor were what led me to try it as a blocker of the strongly H bonding acceptor sites in gallbladder channels.

Probably the value of K_m (equation 1) extracted from Fig. 1 reflects the affinity constant of the binding of TAP with the ligands of the channel. The ligands presumably bind TAP so strongly (compared with their binding to Na^+) that TAP is relatively immobilised and blocks the ligands and channel for Na^+ . This affinity could result from an H bonded association of TAP with the strongly proton acceptor acidic groups of the channel, as illustrated in Fig. 2.

Figure 2 is only an attempt to explain the relatively high affinity of TAP to the acidic ligands of the channel, but other bonds and forces could be important. By studying this interaction in detail, more information on the molecular structure of the channel is expected to be elucidated. The tight junction cationic conductance accounts for most of the passive ionic transepithelial permeability in gallbladder (and probably most leaky epithelia). Because of its specificity and effectiveness in blocking this cation conductance, TAP constitutes a potential tool for the study of epithelial physiology and provides a pharmacological way of specifically controlling the ionic permeability across leaky epithelia.

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Platelet macroglycopeptide

EVIDENCE from electron microscopy^{1,2}, chemical analyses of purified membranes^{3,4} and the use of specific membrane labelling procedures⁵, has demonstrated the presence of a carbohydrate-rich layer on the surface of the blood platelet. This layer contributes many of the groupings⁶ which give rise to the net electronegative charge on the outer surface of the plasma membrane. There is evidence to suggest that the surface-bound carbohydrate is unusual in the platelet both in terms of the large size of the constituent glyco-

protein units⁷ and in the relatively high density of the terminal acidic monosaccharide, sialic acid⁸.

Within the functions of the platelet as a haemostatic cell, various species differences have been shown to exist in terms of the response of the platelet to various stimuli such as ADP, 5-HT and adrenaline⁹. But no differences have yet been shown in the structure of the plasma membrane, elements of which contain the groupings with which external stimuli first come into contact. Here I have investigated the nature of the surface glycoprotein by examining the timed release of acidic glycopeptides from washed platelets by trypsin, followed by their characterisation on polyacrylamide gels. Washed platelets obtained from human, patas monkey (*Erythrocebus patas*) and rabbit blood have been examined in this way. A comparison of the glycopeptides released is shown in Fig. 1.

Trypsin digestion resulted in the rapid release of acidic glycopeptide, as located by Alcian Blue staining, in each of the three species examined. With a digestion time of 1 min, one major band could be seen with only traces of others. After 30 min digestion, however, a more complex pattern was apparent. Two (human), four (rabbit) and six (patas monkey) bands were present, each band representing a glycopeptide, or class of glycopeptides of differing size or charge.

Electrophoresis was performed employing standard 7% acrylamide separating gels¹⁰. Staining capacity was adjudged to be due to complex formation between the Alcian Blue and terminal sialic acid moieties of the constituent oligosaccharide chains of the glycopeptides. The staining capacity was lost following removal of the sialic acid by previous incubation with neuraminidase. It was noticed that after 30 min digestion, the intensity of the first released glycopeptide was much reduced. To ascertain whether the new bands were formed by further digestion of the platelet surface or by hydrolysis of the first glycopeptide, 1 min supernatants from rabbit platelet digests were concentrated, fractionated on Sephadex G-200, and the eluted fractions containing the glycopeptide again subjected to digestion with trypsin for 30 min in conditions identical to those of the original digestion of the washed platelets. After 30 min, a similar

glycopeptide pattern was obtained to that shown in Fig. 1. Thus, it would seem that the later bands are produced as a result of hydrolysis of a single glycopeptide precursor. The major bands as now seen for each species appeared to resist further digestion by trypsin and were still present even after 18 h digestion.

Aliquots of the 1 min supernatants were also subjected to electrophoresis in the presence of the detergent sodium dodecyl sulphate (SDS) using an adaptation of the method of Weber and Osborn¹¹. One major glycopeptide band was seen for each species, the approximate molecular weight of which varied between 168,000 for the rabbit, 147,000 for the human and 142,000 for the patas monkey (Fig. 2).

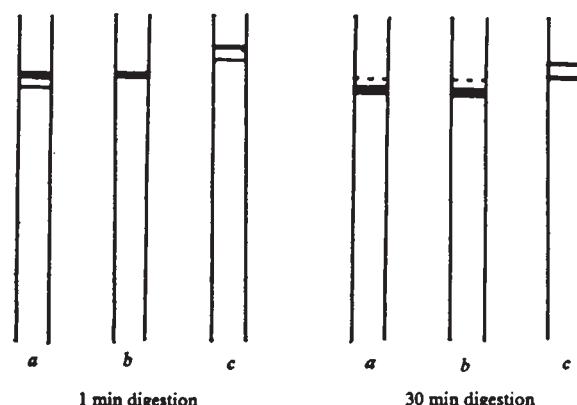


Fig. 2 Aliquots of the trypsin digest supernatants were incubated for 1 h at 37° C in the presence of 1% SDS and 1% 2-mercaptoethanol. Following overnight dialysis against 0.01 M phosphate buffer, pH 7.1, containing 0.1% SDS and 0.1% 2-mercaptoethanol; glycerol and bromophenol blue were added to give final concentrations of 10% (w/v) and 0.02% (w/v) respectively. Aliquots of 0.1 ml were applied to 7% (w/v) polyacrylamide gels containing 0.1% SDS and electrophoresis performed as described by Weber and Osborn¹¹. Glycopeptide was located by the periodate Schiff reaction¹² and molecular weight determinations made in comparison with the relative migration of a series of protein standards¹¹. a, Human; b, patas monkey; c, rabbit.

After 30 min digestion, there was still one glycopeptide band in the human and patas monkey samples, however the approximate molecular weight of this band had fallen to 124,000 and 126,000 respectively. In the rabbit two bands of molecular weight 144,000 and 159,000 were present. In view of possible interference from sulphate groups contributed by the detergent, glycopeptide was here located by the periodate Schiff reaction¹². Substitution of this staining procedure for Alcian Blue in the characterisation of the glycopeptides of the 1 and 30 min digests as illustrated in Fig. 1 showed no alteration in the pattern obtained, indicating a probable absence of large neutral glycopeptides.

No difference in any of the electrophoretic patterns described was seen during examination of a number of individuals of each species. Thus, it would seem that the glycopeptide patterns obtained were characteristic of the species examined. The initial release of a large glycopeptide was common to each, though there were variations in the size of this molecule. Later degradation of the parent glycopeptide by trypsin revealed differences in the number of glycopeptides obtained. This is indicative of differences in the composition of the parent molecule, both in terms of its size and the number of acidic moieties carried. So some differences in the molecular composition of the surface glycoprotein components of platelets of different species are apparent, though the possession of a large parent glycopeptide is common to each of the species examined here. Further investigation of the role of the surface glycoproteins is suggested to determine their role in the reception of external stimuli and in governing the

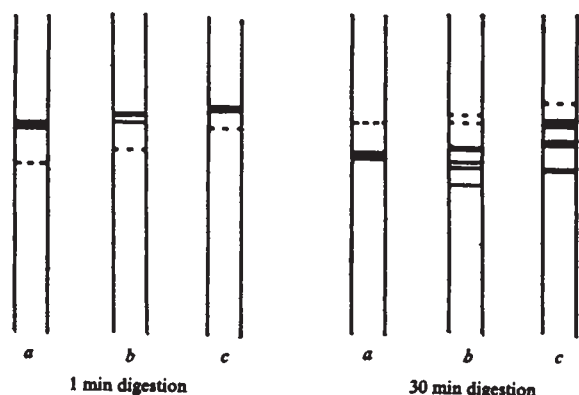


Fig. 1 Washed platelets (2×10^9 ml⁻¹) suspended in 0.01 M Tris-HCl, pH 7.4, containing 0.15 M NaCl, 3 mM EDTA and 0.35% bovine albumin, were incubated in the presence of trypsin (0.2 mg ml⁻¹) at 37° C. At selected time intervals aliquots were removed and added to soybean trypsin inhibitor (0.4 mg ml⁻¹). The platelets were sedimented by centrifugation at 1,500g at 4° C and 0.1 ml aliquots of the supernatant were transferred to 7% polyacrylamide separating gels (0.5 × 7.5 cm) in an even suspension of Sephadex G-25 powder. A small aliquot of bromophenol blue was added as an internal reference standard. Electrophoresis was performed at 200 V (constant voltage) for 1.25 h. The gels were stained overnight in 1% Alcian Blue in 2% acetic acid, destained electrophoretically, and stored in 7% acetic acid at 4° C. a, Human; b, patas monkey; c, rabbit.

permeability of molecules to inner regions of the membrane.

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Amplification of a circular segment of SV40 DNA

DURING serial undiluted passage of simian virus 40 (SV40) in monkey kidney cells, the viral genome undergoes genetic rearrangements involving the incorporation of cellular DNA sequences¹⁻⁵ as well as deletions and reiterations of specific viral DNA sequences^{3,6-8}. In previous work⁷, a mutant of an SV40 variant (DAR; refs 9-11) was identified after serial passage of DAR virus in primary green monkey kidney cells. This mutant DNA was physically mapped by hybridising it to specific DNA segments produced by restriction endonuclease cleavage of SV40 DNA⁸. The mutant DNA was found to contain only one-third of the SV40 genome which was reiterated three times to produce a DNA molecule the size of wild-type SV40 (3.2×10^6 daltons). Most of the early gene sequences and a portion of the late gene region¹² were deleted from the triplicated segment, but it retained the normal site for initiation of viral DNA replication⁸.

The restriction endonuclease from *Escherichia coli*, R-EcoRI, introduces one unique cleavage in SV40^{13,14} or DAR¹⁵ DNA to yield unit length linear molecules with short cohesive termini¹⁶. In the DAR triplication mutant, the R-EcoRI site is symmetrically triplicated; therefore, after R-EcoRI cleavage, segments are formed which are one-third the size of SV40 (DAR) DNA⁷.

In this study, the one-third segments (10.4S DNA) arising after R-EcoRI cleavage of DAR DNA have been isolated. These segments, which contain cohesive termini, have been purified, converted to hydrogen bonded circles and covalently closed by phage T4 polynucleotide ligase to produce circular DNA segments ('minicircles'). We have used the purified 'minicircular' molecules to infect monkey kidney cells in the presence of helper SV40 DNA molecules.

Mertz and Davis¹⁶ have demonstrated that DNA molecules cleaved by endonuclease R-EcoRI can be converted

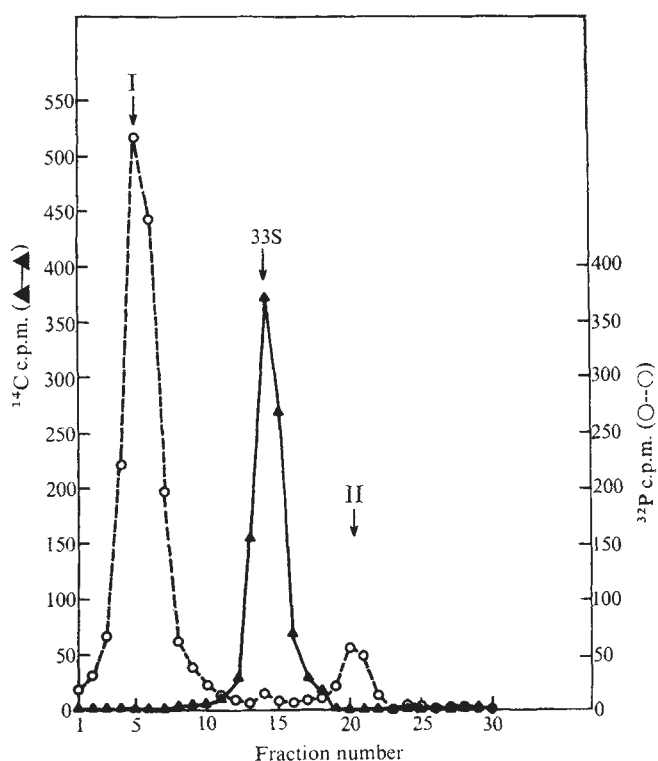


Fig. 1 Analysis of minicircular DNA by alkaline sucrose sedimentation. The minicircular DNA, formed by ligase treatment of 10.4S segments, was mixed with a sample of ³²P-labelled SV40 DNA I (53S) and II (16S) and then sedimented through a 5–20% alkaline sucrose gradient for 130 min at 50,000 r.p.m. in the SW 50.1 rotor¹⁹. DAR ¹⁴C DNA I (ref. 17) was cleaved in a reaction mixture (0.5 ml) consisting of 67 mM Tris-HCl, (pH 7.6), 10 mM MgCl₂, 15 μg of DAR viral DNA, and 0.015 ml of R-EcoRI⁷ (10⁴ units ml⁻¹). After incubation at 37° C for 30 min, the reaction was stopped by chilling to 0° C and adding 10 μmol of EDTA (pH 8.0). The 10.4S DNA segments were isolated by sedimentation through a 5–30% neutral sucrose gradient using the Spinco SW41 type rotor⁷. The pooled fractions were extracted three times with redistilled phenol and dialysed extensively against 0.01 M Tris-HCl (pH 7.5), 0.01 M NaCl, and 0.001 M EDTA at 4° C. The ligase reaction mixture (5 ml) consisted of 66 mM Tris-HCl (pH 7.6), 7 mM MgCl₂, 70 μM ATP, 5 μg of 10.4S DNA and 1.5 units of phage T4 ligase¹⁷. After 16 h at 18° C, additional ATP (0.35 μmol) and ligase (1.5 units) were added and the incubation was continued for 5 additional hours. Then EDTA was added to a final concentration of 14 mM and the minicircular DNA was purified in an ethidium bromide-CsCl density gradient containing 200 μg ml⁻¹ of ethidium bromide¹⁹.

to closed circles *in vitro* because short cohesive termini are created by the staggered nucleolytic cleavage. A DAR¹⁴C-DNA I preparation, containing 80% triplicated DNA, was cleaved with endonuclease R-EcoRI and the 10.4S DNA segments were purified by neutral sucrose rate zonal centrifugation⁷. Minicircles were then formed from the 10.4S linear segments using bacteriophage T4 polynucleotide ligase¹⁷, at a final DNA concentration of 1 μg ml⁻¹ on order to prevent the formation of concatamers and circular oligomers¹⁶. Approximately 80% of the 10.4S segments were covalently closed in the ligase reaction. After the prolonged incubation at 18° C, the covalently closed, circular DNA was purified by dye-density gradient centrifugation. A portion of the heavy band material was then analysed by alkaline sucrose sedimentation (Fig. 1). The minicircles of DAR (SV40) DNA have a sedimentation value of 33S as compared to 53S for superhelical SV40 DNA I molecules. No other DNA species such as dimeric or other oligomeric forms of the 10.4S segments were detectable.

When the CV-1 line of monkey kidney cells¹⁸ was infected with the purified minicircles in the absence of SV40 DNA I, there was no detectable incorporation of ³H-thymidine

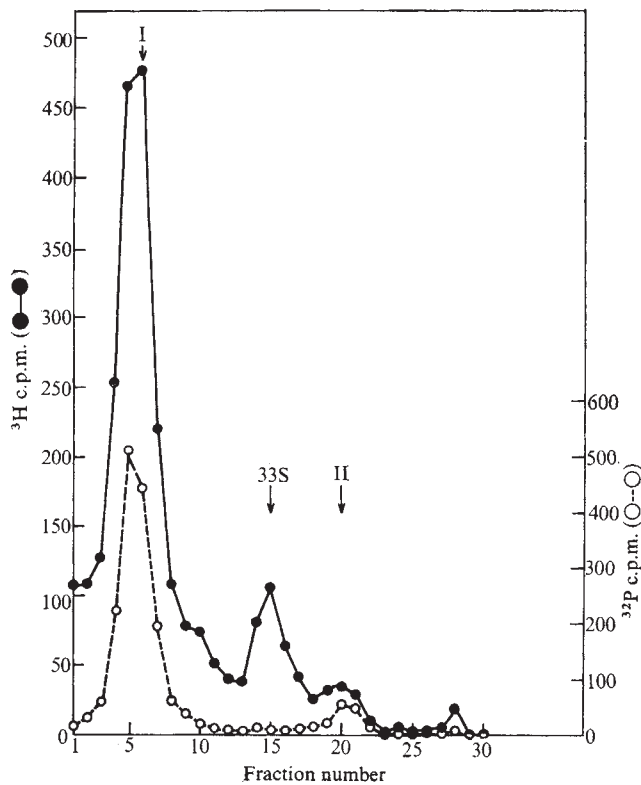


Fig. 2 Alkaline sucrose sedimentation analysis of intracellular DNA I from CV-1 cells infected with SV40 and minicircular DNA. All DNA infections were performed according to the method of McCutcheon and Pagano²⁰. Thus for infection with minicircular ¹⁴C-DNA, or infection with SV40 DNA I and the minicircular DNA together, confluent monolayer cultures of CV-1 cells¹⁸ in 75 cm² plastic flasks (Falcon) were rinsed twice with 20 ml portions of warm PBS (0.008 M Na₂HPO₄, 0.0015 M KH₂PO₄, 0.14 M NaCl, and 0.003 M KCl). The DNA sample (0.8 ml) was then added to 0.4 ml of DEAE-dextran (2 mg ml⁻¹ in PBS) to give a final concentration of 2 µg ml⁻¹ for SV40 DNA, and 0.5 to 1.0 µg ml⁻¹ for the minicircular DNA. For infection with SV40 DNA and the minicircular DNA together, the final concentration of each DNA was the same as in the individual infections. At 60–70 h after DNA infection of CV-1 cells, the medium (Eagle's MEM) was removed and replaced with medium containing 100 µCi ml⁻¹ of ³H-thymidine (6 ml per flask; 50 Ci mmol⁻¹). After 2 h at 37°C, the viral DNA was extracted by the method of Hirt²¹ and the supernatant solution was dialysed against 0.01 M Tris-HCl, (pH 7.5), 0.01 M NaCl, 0.001 M EDTA at 4°C. Superhelical viral DNA was purified by two cycles of equilibrium centrifugation in CsCl-ethidium bromide density gradients and analysed as described in the legend to Fig. 1.

into superhelical molecules banding with SV40 DNA I in dye-density gradients or sedimenting at 33S in alkaline sucrose. This was not unexpected since the 10.4S DNA segments lack most of the early region for SV40 gene expression⁸. In contrast, when cells were infected with approximately equivalent numbers of minicircular and SV40 DNA molecules, ³H-thymidine was incorporated into superhelical molecules having the same density as SV40 DNA I in dye-density gradients (data not shown). When the purified, ³H-labelled, superhelical DNA was analysed by alkaline sucrose sedimentation (Fig. 2), a major peak at 53S and a minor peak at 33S were observed. These results suggest that replication of the minicircular segments requires one or more gene product supplied by the helper genomes.

The composition of the superhelical, ³H-labelled DNA from the mixed infection was further analysed by neutral sucrose rate zonal centrifugation. In Fig. 3a, it is evident that, in addition to molecules sedimenting at 21S (viral genomes of normal size) and at 13.5S (superhelical mini-

circles), a substantial fraction (26%) of the DNA sedimented more rapidly than SV40 DNA I. A portion of this heterogeneous, superhelical DNA preparation analysed in Fig. 3a, was digested with endonuclease R·EcoRI and analysed by neutral sucrose sedimentation (Fig. 3b). Two major bands with peaks at 14.5S (SV40 DNA III) and at 10.4S (one-third segments) were identified. The proportion of molecules (37%) yielding 10.4S segments after cleavage was unexpectedly large considering that the 13.5S band in Fig. 3a comprised only 7% of the labelled DNA. This suggested that part of the 10.4S DNA was present in DNA molecules which were larger than one third of the size of SV40 DNA.

In order to determine whether 10.4S DNA segments had been triplicated or more extensively amplified, different regions of a preparative neutral sucrose gradient were pooled as indicated in Fig. 3a on the horizontal, solid line (pools A–D).

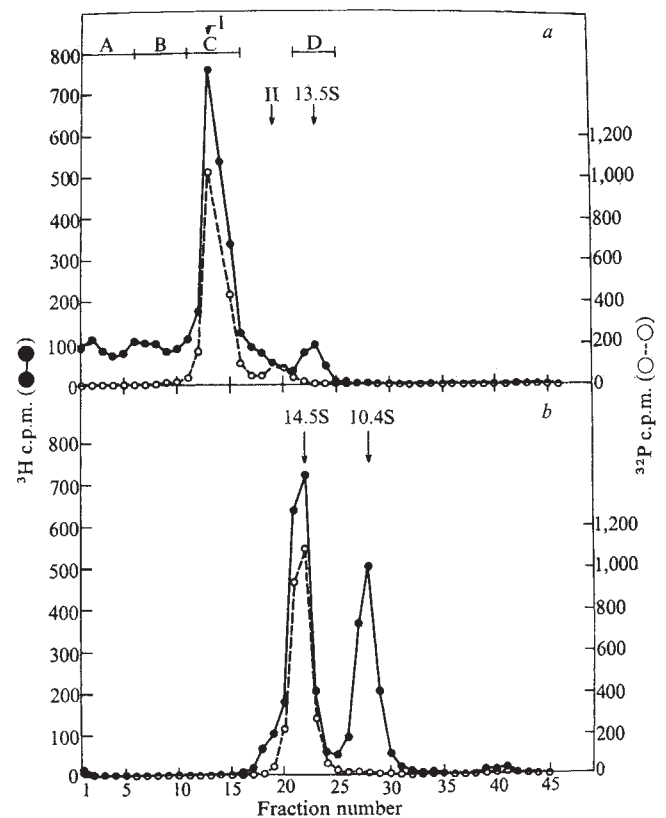


Fig. 3 R·EcoRI cleavage of intracellular DNA I from CV-1 cells infected with SV40 and minicircular DNA. Superhelical viral DNA was isolated as described in the legend to Fig. 2 and analysed¹⁹ by neutral sucrose gradient centrifugation (7 h, 40,000 r.p.m.) in the Spinco SW41 rotor before (a) and after (b) cleavage with R·EcoRI.

Each of these DNA preparations was analysed separately before and after R·EcoRI cleavage by neutral sucrose sedimentation (Fig. 4). Fractions from pool A (Fig. 4a) comprised 12% of the total superhelical DNA and had an average sedimentation value of 27.6S (molecular weight = 5.7×10^6 ; ref. 22). After R·EcoRI cleavage (Fig. 4d), a peak at 10.4S (51% of pool A) was present with two less well defined peaks at 14.5S and at 16.7S. Pool B (Fig. 4b) had an average sedimentation value of 25.3S (molecular weight = 4.7×10^6) and, after cleavage (Fig. 4e), produced molecules which sedimented at 14.5S (25%) and at 10.4S (64%). After cleavage of pool C, which sedimented with SV40 DNA I (21S), 78% of this DNA sedimented at 14.5S while 18% had a sedimentation value of 10.4S. Pool D (data not shown) produced only 10.4S DNA after cleavage, as ex-

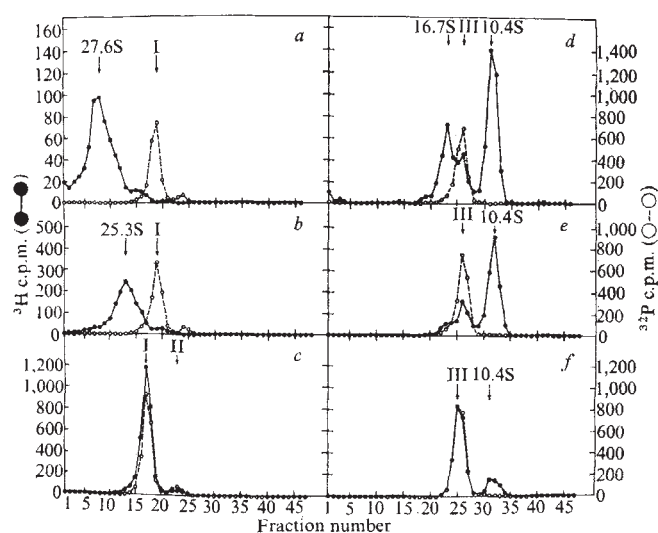


Fig. 4 Analysis of partially fractionated oligomers isolated from cells infected with SV40 and minicircular DNA. Different regions of a preparative neutral sucrose gradient similar to Fig. 3a were pooled as indicated in Fig. 4a on the horizontal solid line. Each of these DNA preparations was then analysed separately before and after cleavage by R-EcoRI as described in the legend to Fig. 3. The analyses of pool A are shown in a (before cleavage) and d (after cleavage), pool B in b and e and pool C in c and f.

pected. These results show that molecules of much higher molecular weight than the minicircular DNA are a source of 10.4S DNA after R-EcoRI cleavage and suggest that amplification of the 10.4S segments had occurred producing circular oligomers of this segment.

When the mixed infection of CV-1 cells with minicircular and SV40 DNA was allowed to proceed to completion, the virions purified from the lysate formed only one band in a CsCl density gradient¹¹. DNA extracted from the purified virions sedimented in a narrow band in a neutral sucrose gradient at the position of SV40 DNA I (data not shown). Thus, only molecules having the same size and shape as SV40 DNA were encapsidated. After R-EcoRI cleavage of the DNA from virions, molecules sedimenting at 14.5S and 10.4S were found, indicating that trimeric genomes had been encapsidated. Furthermore, cleavage of these 10.4S segments with the restriction endonuclease from *Hemophilus influenzae* produced five fragments having the same electrophoretic mobility as those produced from the original 10.4S DNA⁸.

These results indicate that circular, duplex viral DNA segments much smaller than SV40 DNA can be replicated *in vivo*. In addition they support the model proposed by Khoury *et al.*⁸ for the generation of the original DAR triplication mutant. In our experiments, cells were infected with a minicircular DNA molecule, formed *in vitro*, which then served as a precursor *in vivo* in the formation of trimers and higher oligomers as predicated by the proposed model. The DAR triplication mutant first appeared after the third passage in primary monkey kidney cells and rapidly became the predominant species in later passages⁷. This mutant may have had a strong selective advantage since it contained three sites for initiation of DNA synthesis. The oligomeric forms in our studies may have evolved for a similar reason.

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Leukaemogenic effect of repeated inoculations with small doses of Tennant virus in BALB/c mice

DOSE-RESPONSE studies of oncogenic viruses in animals are usually restricted to single inoculations. The study of the effect of dose fractionation has been a rather neglected subject in this field. An important question is whether the combined effect of the small doses is less than the effect of a single exposure to the same total dose. In this study on dose fractionation of a murine leukaemia virus we observed the opposite effect.

The B/Tennant leukaemia virus was isolated from a spontaneous lymphosarcoma of BALB/c mice¹. It usually induces this disease in BALB/c mice with a relatively short latency period¹⁻³. Dr J. Tennant (Sloan-Kettering Institute, New York) sent us a sample of the virus in 1969 and we then passed it continuously by inoculations of crude cell-free extracts of leukaemic tissues into neonate BALB/c mice. For the present study, 1 l of a 10% cell-free extract was prepared as previously described⁴ and stored in liquid nitrogen in 10 ml aliquots. Intraperitoneal inoculations with virus were started in female BALB/c mice (20 per group) when they were six weeks old.

Table 1 Leukaemia incidence and latency in BALB/c mice repeatedly injected with Tennant murine leukaemia virus

Virus dilution	Number of inoculations					As many as possible till autopsy
	1	3	6	12		
10^{-1}	100 (44±8)*	100 (43±5)	100 (38±10)	100 (41±8)	100 (35±4)	
10^{-2}	100 (49±5)	100 (49±6)	100 (47±6)	100 (43±5)	100 (47±7)	
10^{-3}	100 (80±18)	100 (67±22)	100 (59±11)	100 (58±15)	100 (49±6)	
10^{-4}	35 (109±3)	20 (82±14)	75 (79±19)	100 (64±20)	100 (56±13)	
10^{-5}	0	0	25 (121±18)	65 (74±14)	100 (69±20)	
10^{-6}	0	0	0	80 (99±8)	100 (72±11)	

* %Incidence (latency period (d) ± s.d.).

† The average number of injections in these series is 15, 19, 20, 22, 28 and 29 respectively.

The mice were injected three times a week. They were examined twice a week for splenomegaly and/or swollen lymph nodes. The animals were then autopsied and histologically examined for confirmation of the development of leukaemia. The survivors were kept under observation only up to the age of 10 months. After that age, the first spontaneous lymphoreticular neoplasms begin to appear in this strain.

From the results in Table 1, it is obvious that a 1,000-fold dilution is still fully leukaemogenic. Higher virus doses or repeated inoculations tend to shorten the latency period. There is no indication that dose fractionation leads to a lower incidence or longer latency.

In fact we found that repeated inoculations with low doses of virus produce a significantly higher leukaemia incidence than a single injection with a much higher dose (for instance, 12×10^{-6} instead of 1×10^{-4}). A possible explanation is that continuous exposure to even very small numbers of virus particles induces immunological unresponsiveness to the virus, so that these small numbers may be propagated in the host. Alternatively, autointerference induced by a moderate dose of virus may play an important part.

It would be worthwhile to investigate whether a similar procedure can overcome the genetic resistance of some mouse strains to the virus^{2,5}, as well as that of adult rats. The observation must be extended to other tumour virus systems. If it proves to be of a general nature, it has important biohazard implications for tumour virological workers or investigators handling cell cultures which produce small amounts of C-type oncornavirus particles⁶.

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(CHO) cells have shown that after two rounds of replication in the presence of BrdU or IdU, sister chromatids stain differentially with Giemsa, allowing the identification of the two chromatids, and the observation of sister chromatid exchanges (SCEs) without recourse to autoradiography. The chromatid with the bifilarly substituted DNA (BrdU substituted in both strands of DNA) is less condensed and stains more weakly with Giemsa than the unifilarly substituted sister chromatid. The yield of SCEs is approximately that observed by autoradiography.

Latt⁴ has described a fluorescence technique for human chromosomes using the dye Hoechst 33258. This dye normally fluoresces intensely when bound to poly(dA-dT), but when



Fig. 1 CHO cell stained with Hoechst 33258 after two rounds of replication in the presence of BrdU. Fluorescence performed as in ref. 4. Leitz ortholux microscope, 200 W mercury light source UG-1 filter and TK400 dichroic mirror for excitation, and K400 and K430 barrier filters.

New Giemsa method for the differential staining of sister chromatids

If human lymphocytes¹ or Chinese hamster² cells are treated with the base analogue 5-bromodeoxyuridine (BrdU) in the latter part of the S period, Giemsa stained chromosomes exhibit a pattern of condensed and extended segments along their length. This phenomenon has been attributed to a delay in the spiralisation pattern of the late replicating regions along the chromosomes. Other experiments³ with Chinese hamster ovary

bound to poly(dA-BrdU) the fluorescence of the dye is quenched. After two rounds of replication in the presence of BrdU, followed by staining in Hoechst 33258, the unifilarly substituted chromatids fluoresced more brightly than the bifilarly substituted sister chromatids. Sister chromatid exchanges were readily observed. Though this technique provided a degree of resolution for the detection of SCEs which was previously not possible, the dye demonstrated a remarkable degree of photoinstability, so that the image faded very rapidly. This paper describes a technique in which the use of Hoechst 33258 is combined with Giemsa staining (fluorescent plus

Giemsa or FPG) to make permanent Giemsa-stained cytological preparations of differentially labelled chromatids.

Chinese hamster ovary (CHO) cells were cultured in McCoy's 5A medium supplemented with 13% foetal calf serum. Exponentially growing cells were treated with BrdU (final concentration 10 μ M) for 24 h during which time two rounds of replication occurred.

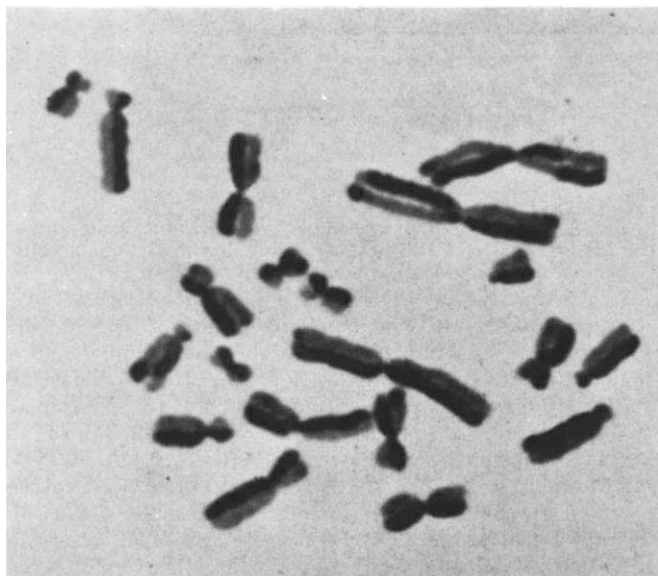


Fig. 2 Cell stained with Hoechst 33258 and then with Giemsa.

All cultures containing BrdU were kept in the dark to minimise the numbers of sister chromatid exchanges caused by the photolysis of BrdU containing DNA³. Colcemid (final concentration 2×10^{-7} M) was then added for 2 h and the mitotic cells collected by shaking. The cells were treated for 8 min with 0.075 M KCl to spread the chromosomes and then fixed in methanol acetic acid (3:1).

Drops of cells in fixative were placed on clean microslides and allowed to dry. The slides were then stained in Hoechst 33258 (0.5 μ g ml⁻¹ deionised water) for 12 min and rinsed briefly in deionised water. The preparations were mounted in water and the cover slip ringed with Holdtite rubber cement to prevent evaporation. Sister chromatids now fluoresced differentially (Fig. 1) as Latt has reported⁴.

These same preparations were then allowed to age for 24 h, after which time the coverslips were removed and the slides incubated for 2 h at 60° C in either $2 \times$ SSC (0.3 M sodium chloride-0.03 M trisodium citrate) or in water. This was then followed by 30 min staining in 3% Giemsa solution (Gurr's R66 pH 6.8). If SSC was used G bands could sometimes be seen.

Figure 2 shows a cell after Hoechst 33258 and Giemsa staining. The Giemsa preparation is now permanent, that is does not fade rapidly, and can be studied at leisure with the compound microscope. The chromatid that incorporated BrdU into both strands of the DNA helix fluoresced dimly and now stains weakly with Giemsa whereas the chromatid that incorporated BrdU into only one strand fluoresced brightly and now stains darkly with Giemsa.

Latt⁴ has suggested that the difference between the two chromatids is caused by a differential quenching of fluorescence by the BrdU incorporated in either one or two strands of the DNA. Ikushima and Wolff⁵, however, have postulated that differences in the binding of protein to BrdU-containing chromatids is the cause of the differential staining with Giemsa. The differential Giemsa stainability observed here of chromatids pretreated with Hoechst 33258 indicates that the difference cannot be attributed simply to quenching of fluorescence.

Table 1 Sister chromatid exchanges (SCE) observed in CHO cells after two rounds of replication in the presence of BrdU

Stain	(No. of SCE)/ (No. of chromosomes)	No. of SCE per chromosome
Hoechst 33258	360/554	0.65 ± 0.034
Hoechst 33258 followed by Giemsa	149/223	0.668 ± 0.055

This differential staining of the two sister chromatids can also be observed if the cells are stained for 5 min with 0.005% acridine orange in Sørensen's buffer at pH 6.7, but is only barely seen, if stained with quinacrine. The difference in fluorescence between the two chromatids, however, is not readily distinguished until the cells are exposed to exciting light passed through a BG 12 filter. Only those cells so exposed show a subsequent differential staining of sister chromatids with Giemsa. If, however, too much exciting light falls on the cell and too much fading of the fluorescence occurs, then the subsequent Giemsa staining is very weak. Similarly, if cells stained with Hoechst 33258, which fades very rapidly, are exposed to exciting light they ordinarily do not stain as well with Giemsa as unexposed cells.

The number of SCEs observed when cells are stained with Hoechst 33258, with or without subsequent Giemsa staining, is greater than the number observed after staining in Giemsa alone³ or after autoradiography⁶. In addition all chromosomes in a cell can be scored whereas after autoradiography only the larger chromosomes are scorable.

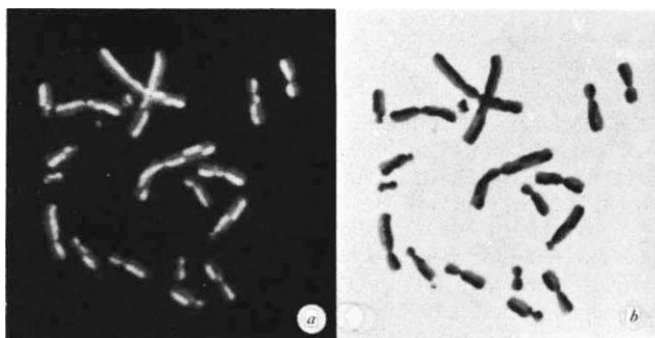


Fig. 3 Cell stained first with Hoechst 33258 (a) and subsequently with Giemsa (b).

Table 1 presents results of an experiment in which cells were grown in 5 μ M BrdU. The frequency of sister chromatid exchanges observed in 33258 plus Giemsa-stained cells was the same as that observed in cells stained with 33258 alone. Furthermore, in Fig. 3 it may be seen that the same exchanges are observable by each technique. The FPG technique does seem to offer some additional resolution, however. We have noticed that when small double exchanges occur near the tips of chromosomes, these can be seen clearly with Giemsa, whereas after fluorescent stain alone, because of flare, both tips often appear to fluoresce and thus mimic isolabelling as seen in autoradiographic preparations.

It therefore seems that the technique presented here has all of the advantages of the fluorescent technique discovered by Latt, but has the additional advantages of producing nonfading permanently stained cells that do not require fluorescence microscopy and photography for their study.

We thank Dr H. Loewe of Farbwerke Hoechst for the Hoechst 33258.

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Effect of cysteine on the survival of mice with transplanted malignant thymoma

TISSUE collagenase has been detected in inflamed human tissues by Fullmer and Gibson¹, and in some malignant neoplasms by Riley and Peacock². The possible role of tissue collagenase in neoplastic spread has been suggested by Birbeck and Wheatley³ from an electron microscopic study of the invasion of ascites tumour cells into the abdominal wall of BALB/c mice. They reported that the tumour cells appeared to be actively destroying adjacent normal tissues, which is suggestive of the activity of tissue-destroying enzymes derived from the neoplastic cells. The finding of collagenase activity in human and animal tumours by Riley and Peacock², Campbell⁴, and Dresden, Heilman and Schmidt⁵, prompted a study into the effect that collagenase inhibitors might have on the spread of neoplastic disease. It has been reported by Brown and Weller⁵ that corneal ulcerations which inevitably occur following severe alkali burns are due to endogenously produced collagenase and that they can be prevented by topical application of solutions of collagenase inhibitors such as cysteine. The present investigation was designed to test our hypothesis that collagenase produced in malignant neoplasms is a factor in neoplastic invasion and that by inhibiting its activity the arrest of a malignant neoplastic disease could be achieved. The results reported here of initial experiments demonstrate the beneficial effects of cysteine on the survival rates of animals into which malignant thymomas were transplanted.

The malignant thymoma was originally obtained from the Walter and Eliza Hall Institute, Melbourne, where it had been repeatedly transmitted in C57Bl mice. Fresh specimens of thymoma, which have been shown in our laboratories to produce collagenase on *in vitro* cultivation, were cut into 1 mm³ and then dispersed into single cell suspensions by teasing in sterile 0.15 M NaCl.

Table 1 The effects of cysteine on the survival rates of animals after transplantation of malignant thymomas

Treatment	Mean survival time (d)	Weighted mean survival time (d)
Thymoma alone	31	31
Thymoma + 0.15 M NaCl	34	45
Thymoma + 0.01 M cysteine	37	37
Thymoma + 0.1 M cysteine	37	37
Thymoma + 0.25 M cysteine	65*	98
Thymoma + 0.5 M cysteine	58	86
Thymoma + 1.0 M cysteine	54	65

* Student's *t* test significant at the *P* < 0.05 level.

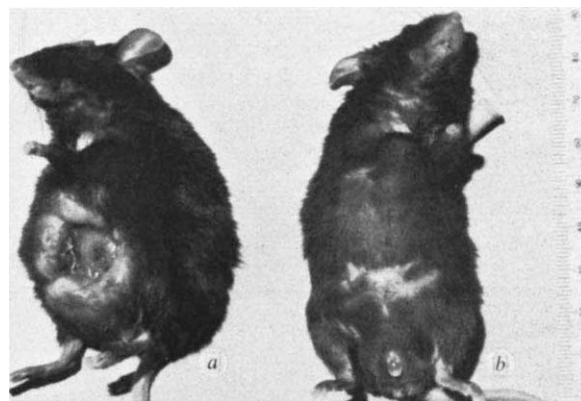


Fig. 1 *a*, C57Bl mouse injected with thymoma cells alone died at 38 d. *b*, C57Bl mouse injected with thymoma cells + 0.25 M cysteine and killed at 100 d.

Approximately 10⁷ viable cells in 0.3 ml of 0.15 M NaCl were infused subcutaneously into the abdominal tissues of male C57Bl mice weighing 20–25 g. The study was designed so that there were seven groups, each with six animals, treated as shown in Table 1. For 8 d, 0.2 ml of the varying concentrations of cysteine; 0.01 M, 0.1 M, 0.25 M, 0.5 M and 1 M; and 0.15 M NaCl as a control, were infused as near as could be determined to the same site as the depot of thymoma cells, commencing 10 d after the initial inoculation of thymoma cells. The macroscopic difference between mice injected with thymoma cells and given no therapy, as illustrated in Fig. 1*a*, or given 0.2 ml of 0.25 M cysteine daily for eight days, as illustrated in Fig. 1*b*, is clearly evident.

The results of the experiment are illustrated in Table 1 and show that 0.25 M cysteine injections significantly (*P* < 0.05) prolonged survival time as compared with the saline treated controls and to higher and lower doses of cysteine. The weighted mean (Table 1) takes into account the number of surviving animals at any particular point of time and was calculated according to the method described by Zippin and Armitage⁶ using 100 d as the end point of the experiment and highlights in these experiments the difference between control and experimental animals, especially those in which 0.25 M cysteine was administered.

The results of this study suggest that 0.25 M cysteine, introduced locally, is the most effective concentration to inhibit the growth of transplanted malignant thymomas in C57Bl mice.

If the effect of the cysteine is due to its collagenase inhibiting effect described in the literature by Slansky and Gnadinger⁹ and Brown and Weller¹⁰, and if the limitations of tumour growth in the described experiments was due to this effect, an important area of investigation is suggested because of the known ability that some malignant neoplasms have of producing tissue collagenase.

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Occurrence of lead in tuna

At a recent national conference in the United States on baseline studies of pollutants in the marine environment¹ serious doubts were raised about the validity of most published measurements of lead concentrations in seawater and in marine organisms. Available data for foods and other plant and animal tissues are also implicated because they may contain similar concentrations of lead.

It was recommended at the baseline meeting that a workshop be established to carry out interlaboratory analyses of lead in seawater. The workshop, sponsored by the National Science Foundation Office for the International Decade of Ocean Exploration (IDOE), was initiated in the autumn of 1972. Samples of seawater standardised at one university by isotope dilution were circulated among participating oceanographic laboratories at seven universities in the United States and at one ministry in the United Kingdom. None of the participants was aware of the standardised value until all results had been reported. Results of the workshop confirmed suspicions that not one oceanographic laboratory of superior status could, as of September 1973, collect and analyse lead in coastal surface seawater by atomic absorption or anodic stripping voltammetry techniques and report reliable values^{2,3}. It seems that most published measurements of lead in seawater may be erroneously high by factors of 10 to 100.

We have now determined lead concentrations in tuna fish by a reliable analytical technique. The observed lead concentrations in muscle are four orders of magnitude lower than those in epidermis. Both values lie so far away from generally accepted levels of lead in tissues that analysts may find it difficult to recognise that such lead concentrations actually exist. These new findings strongly suggest, as in the case of seawater, that recent studies of the hazards of lead pollution may be misleading if they are based on analyses of plant and animal tissues determined by routine analytical methods carried out without

the use of clean-laboratory techniques and without the necessary sensitivity and accuracy.

The lead in tuna data are presented here as frames of reference for investigators applying atomic absorption or potentiometric analytical methods to studies of lead in plant and animal tissues.

Our data were determined in two laboratories by stable isotope dilution using thermal ionisation source high resolution mass spectrometers and special clean-laboratory chemical techniques^{2,4}. We analysed five types of samples: (i) tuna muscle uncontaminated with lead by handling, and containing such small concentrations (~0.0003 p.p.m. wet) that they must be determined with special care; (ii) tuna epidermis, where lead is found concentrated to levels (~2 p.p.m. wet) that are poisonous to humans; (iii) tuna scales and dermis, which contain much less lead than epidermis; (iv) commercial canned tuna muscle, which is contaminated with industrial lead by a factor of 1,000 above levels in living tuna; and (v) a tuna muscle interlaboratory reference that could not be analysed properly by investigators using atomic absorption and anodic stripping techniques in oceanographic laboratories because it did not contain sufficient lead (~0.4 p.p.m. dry), even though it had been contaminated with industrial lead by a factor of 400 above levels in living tuna during preparation by a commercial food manufacturer.

Frozen tuna were obtained from IDOE. The fish were thawed at 4° C for 24 h, and then exposed to room temperature for 1 h, leaving them still partially frozen and suitable for dissection. Clean-room procedures were used to prevent industrial lead contamination, but additional precautions had to be taken to minimise transfer of mucosal slime containing large amounts of lead to interior tissues during dissection.

Water was removed from tissues either by heating to 110° C or by vacuum desiccation. Values for water contents are approximate because of dehydration and plasma losses between the times when the fish were caught and the thawed tissues were weighed. Three methods for decomposing tissue for analysis were used in two laboratories: muffle furnace oxidation of 15–40 g wet tissue samples was used at the Scripps Institution of Oceanography; and either low temperature ashing with an excited oxygen plasma or wet acid oxidation of 0.5–4 g wet tissue samples were used at the California Institute of Technology. Weighed amounts of lead isotope tracer were homogenised with the lead in the samples during digestion and oxidation; the lead isotope mixtures were isolated by ion-exchange and solvent extraction, and were then analysed in a mass spectrometer. The amounts of lead in the samples were calculated from the observed ratios of ²⁰⁷Pb_{sample} to ²⁰⁸Pb_{tracer} and the weights of tracer. Concentrations were calculated from sample weights.

Results for tuna analyses are listed in Table 1. Observed lead concentrations in tuna muscle were so small there was

Table 1 Lead in tuna

Sample Type	Tissue	p.p.m. Pb (wet)	wt % H ₂ O
8 kg Yellowfin (<i>Neothunnus macropterus</i>)	Pectoral fin	5.3	42
8 kg Skipjack (<i>Katsuwonus pelamis</i>)	Pectoral fin	4.1	38
7 kg Albacore (<i>Thunnus alalunga</i>)	Pectoral fin	0.45	32
(oxidise in excited oxygen plasma)	Epidermis	1.7	67
	Epidermis	1.4	67
	Scales	0.06	25
	Dermis	0.01	44
(acid digest at atmospheric pressure)	Gill filaments	0.014	73
	Muscle	0.0002*	71
	Muscle	0.0003*	71
(acid digest at high pressure in bomb)	Muscle	0.0003*	71
Canned tuna (in oil) cannery A	Muscle	0.10	57
Canned tuna (in oil) cannery B	Muscle	0.93	57

* Uncertainty is ~30% for these values. Concentrations are three times larger than the present limit of measurement for a 4 g sample, which is set by the total analytical blank. At this time this blank is 1.8 ng Pb.

Table 2 Lead in US NBS reference materials by three different decomposition methods

Reference material	Muffle furnace (SIO)	Excited oxygen plasma and wet acid (CIT)
Bovine liver (as received)	0.333 (20% spread)	0.328 (5% spread)
Tuna muscle (vacuum dried)	0.22 to 0.35	0.34 to 0.45

All figures are expressed in p.p.m., and are means from multiple analyses
SIO, Scripps Institution of Oceanography; CIT, California Institute of Technology

concern whether proper mixing of tracer and sample lead isotopes had been achieved. Results obtained by hot HNO_3 - HClO_4 decomposition at atmospheric pressure were checked by carrying out parallel acid decompositions in Teflon bombs at 180°C and at very high pressures. No significant differences in lead concentrations were observed between the two methods, so we think that proper isotopic equilibration had been achieved at ordinary temperatures and pressures. It seems likely that fresh tuna muscle, uncontaminated by handling, contains about 0.0003 p.p.m. Pb. This contrasts with the very high concentrations of lead in epidermis, which are about 2 p.p.m. in wet tissue. Humans are poisoned if they ingest food containing several p.p.m. Pb for a prolonged period.

It is doubtful that this excessive epidermal lead is an artefact derived from fishing operations because, as shown in Table 1, we have found similar concentrations in whole pectoral fins from different tuna obtained under various circumstances. On a mass balance basis, the lead content of fish fins is determined almost entirely by the concentration of lead in epidermis. The mucin secreted by the mucous cells of the epidermis contains a glycoprotein which reacts with water to form mucous slime. It is likely that strong heavy metal complexing sites in these proteins withdraw lead from seawater and incorporate it into the slime. Our samples of epidermis included the slime coating, and we believe that most of the lead in tuna epidermis is located in epidermal slime. Coombs *et al.*⁵ have shown that Ca and Zn are probably concentrated from seawater into epithelial mucus.

Muscle contains quite small concentrations of metabolised lead, probably because of biochemical exclusion mechanisms in the gut membrane. As Table 1 shows, mucus on epithelial tissues in gill filaments do not contain high concentrations of lead, so that it does not seem likely that much lead is adsorbed by gill mucus from seawater and metabolised into fish muscle from gills. As shown in Table 1, for tissues immediately underlying the epidermis, lead concentrations in scales were $1/26\text{th}$ of that in epidermis, while lead in dermis was $1/150\text{th}$ of that in epidermis, but these values may be erroneously high because of cross contamination from mucosal slime during dissection. It seems unlikely that much lead passes through the skin barrier from seawater.

Measured lead concentrations in several sample of commercial canned tuna are listed in Table 1. There is a considerable range in values, one being clearly dangerous to human health. The levels of lead in canned tuna available for human consumption are about 1,000 times above lead levels existing in living tuna. The following evidence shows that this difference in lead levels is common, and is due to contamination by industrial lead.

An interlaboratory reference made of selected high grade fillets of muscle from albacore was prepared by the US National Bureau of Standards (US NBS). The frozen meat fillets were shipped to a commercial food processor, where they were dehydrated, ground, and mixed. Aliquots were placed in polythene bags, and these bags were sealed with lead-soldered vacuum cans for distribution. Lead concentrations determined in this tuna muscle reference are listed in Table 2, together with results obtained for the US NBS bovine liver reference. We consider the bovine liver

to be homogeneous in lead on a 0.3 g scale and claim it contains lead within $\pm 2\%$ of the values reported in Table 2 irrespective of the certified value reported by the US NBS (the certified US NBS value is a mean of values determined by different methods, some of which are less reliable than isotope dilution for lead at these concentrations). The considerably wider spread in values obtained for the albacore muscle reference suggests that it is not homogeneous in lead on a 0.3 g scale. The albacore that made up this reference were caught in exactly the same manner, time, and place as the albacore listed in Table 1 (off the southern California coast, July, 1971). The fact that the commercially handled muscle contains 400 times more lead than the same muscle dissected under clean conditions, together with the heterogeneous occurrence of lead in the commercially handled muscle, shows that the tuna muscle reference was contaminated by industrial lead in a commercial food factory by a factor of 400 after it was removed from the sea. This justifies the assertion that canned tuna, representative of that available for human consumption, is contaminated by industrial lead to the extent of about 1,000 times above lead levels in living tuna.

Mucosal slime is not the source of the major part of this lead contamination because it could only raise muscle concentrations by a maximum factor of about 20. Mass balance observations of the distribution of lead in most organs of tuna show that 3% of all the lead in tuna resides at a concentration of about 0.0003 p.p.m. in fresh muscle, while 52% resides in the epidermis at a concentration of about 2 p.p.m. Muscle comprises about 75% of tuna body weight, while epidermis amounts to about one quarter of 1%. The total body burden of lead in albacore tuna is about 0.008 p.p.m.

The following evidence (from ref. 1) suggests that it is difficult, if not impossible, at present for most analysts to determine reliably true values of lead concentrations in marine organisms. The US NBS orchard leaf, bovine liver and tuna references were sent by IDOE for metal analyses to eleven laboratories participating in the IDOE Baseline Studies of Heavy Metals Program. All analysts using atomic absorption and anodic stripping voltammetry techniques were willing to report approximately correct values for the orchard leaf reference, even though it was irrelevant to the problem of analysing lead in marine organisms, because the answer was confidently known and because it contained one hundred and fifty thousand times (44 p.p.m.) more lead than does tuna muscle dissected in a clean laboratory. Not one reported an erroneous value. About half of these analysts refused to report a lead value for the bovine liver reference, while the remainder reported that the lead concentration was less than their ability to measure it (~ 0.5 p.p.m.). Not one reported an erroneous value. These analysts knew with confidence that lead was about 0.3 p.p.m. in this reference. Most of these analysts refused to report anything regarding lead in the tuna reference. A few reported that lead was less than their measurement limit (~ 0.5 p.p.m.), while a few reported erroneous lead concentrations that were about an order of magnitude too high. The analysts did not know, before they analysed it, that the lead concentration was 0.4 p.p.m. in the tuna reference.

Since marine organisms can possess soft tissues in close

juxtaposition which contain lead concentrations that differ by a factor of 5,000, and since the major mass of live tuna tissue contains such low concentrations of lead that it can be polluted with lead by a factor of 400 before analysis and still contain less lead than most analysts can measure at present, it is clear that a problem exists regarding the evaluation of lead pollution in the marine environment. These difficulties probably extend to the terrestrial environment as well, since it has been recently shown that native field mouse muscle contained only 0.0015 p.p.m. lead in an animal that was known to be 83% polluted with industrial lead, and that mountain freshwater streams contain two orders of magnitude less lead than can be reliably measured by most analysts at present⁸.

A fresh outlook regarding lead analyses of biological materials is required. The practical sensitivities and accuracies of atomic absorption and anodic stripping techniques do not appear to be the main sources of difficulty². The real problem appears to be a pervasive lack of understanding of the extent, sources, and ways to eliminate laboratory lead contamination. Substantial improvements could be made if analysts devoted much more care and attention to the evaluation of analytical lead blanks and their reduction and control by the institution of clean-laboratory procedures, together with a realistic scaling up of the quantities of sample lead involved in an analysis so as to substantially exceed laboratory analytical lead blanks.

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potential modality for immunotherapy of human cancer. Several preliminary clinical trials have been reported in which 'educated' lymphoid cells were administered to cancer patients^{9,10}, although the clinical benefits of this procedure remain uncertain. We describe here culture conditions that increase cytotoxic activity of melanoma patients' lymphocytes against melanoma-derived target cell lines.

Dr Hector Sulit of our laboratory established target cell lines from three patients with malignant melanoma and from one patient with osteosarcoma. Control fibroblast lines were established from uninvolved areas of skin or muscle from these patients. The lymphocytes and the cultured cell lines were designated as follows: lymphocytes as 4L, 10L and 14L; cell lines as MLA4, MLA10 and MLA14; fibroblast cultures as MLA4F, MLA10F and MLA14F; and the sarcoma target cell culture as SLA1. The long term breast carcinoma line, BT20, was used in experiments 2 and 4. All other cell lines were used between the fifth and fifteenth passage. All cell lines were free of detectable mycoplasma contamination.

Lymphocytes, usually containing 5-15% monocytes and less than 2% granulocytes, were prepared from peripheral blood samples by Ficoll-Isopaque gradients and were stored in viable condition by slow freezing. For the sensitisation procedure, $2-4 \times 10^6$ tumour cells were allowed to form a monolayer in a 25 cm² Falcon tissue culture flask. Once the cells were firmly attached, the monolayers were treated with $5 \mu\text{g ml}^{-1}$ of mitomycin C and incubated for 18 h. The monolayers were washed and 5×10^6 to 9×10^6 lymphocytes were added in RPMI 1640 medium supplemented with antibiotics, 15 mM Hepes buffer and 20% pooled normal AB serum. The lymphocytes were sensitised on the monolayers for 5 d, after which they were recovered by washing with warm media. Viable lymphocytes were counted by trypan blue exclusion in a haemocytometer (tumour cells were not viable at this point due to mitomycin). Forty to sixty per cent of the original lymphocytes in culture could usually be recovered. The lymphocytes were washed and resuspended at 1.0×10^6 to $1.5 \times 10^6 \text{ ml}^{-1}$. Three or more replicate samples of 0.1 ml were tested for ³H-thymidine incorporation and additional aliquots were tested for cytotoxicity. Lymphocyte proliferation was determined by a micro-MLC assay similar to that of Thurman *et al.*¹¹. Samples were pulsed with 0.5 μCi of isotope and incubated for 18 h before collection. Cytotoxic activity was assayed by the ¹²⁵I-iododeoxyuridine (IDU) method of Cohen *et al.*¹². In this test, lymphocytes are added to ¹²⁵I-IDU-labelled target cells in Falcon Microtest II plates, usually at an effector: target cell ratio of 100:1 (some experiments involved ratios of 150:1 and 50:1). After 40 h of incubation, the plates were washed to remove lymphocytes and dead target cells. The c.p.m. of isotope remaining in each well indicated the number of target cells surviving the interaction with the lymphocytes.

All experiments had a control culture of lymphocytes cultured for 5 d in the absence of a stimulator cell. Results of the ³H-thymidine incorporation are expressed as a response index (RI) calculated by the formula $RI = \text{Average c.p.m. of sensitised lymphocytes} / \text{Average c.p.m. of control lymphocytes}$.

Results for the cytotoxicity assay are expressed as a cytotoxic index (CI) calculated by the formula $CI = [(\text{average c.p.m. in wells} + \text{control lymphocytes}) - (\text{Average c.p.m. in wells} + \text{sensitised lymphocytes})] / [\text{Average c.p.m. in wells} + \text{control lymphocytes}]$.

By calculating the results in this fashion, we could describe only the cytotoxic activity obtained by the sensitisation procedure, rather than the cytotoxicity intrinsic to the melanoma patient's lymphocytes.

In experiment 1, the lymphocytes from melanoma patient 14 were sensitised *in vitro* on autochthonous and allogeneic tumour cells (Table 1). Autochthonous and allogeneic sensitisation produced a small, but significant increase in lymphocyte proliferation. Both sensitised lymphocyte populations displayed increased cytotoxicity against melanoma target cells,

Sensitisation of lymphocytes *in vitro* against human melanoma-associated antigens

SENSITISATION of lymphocytes *in vitro* facilitates the study of cellular immune mechanisms under conditions in which the exact input of reactive cells and antigen is controlled. Such systems have been used to generate cytotoxic effector cells against alloantigens¹⁻³, and more recently against tumour-associated antigens (TAA) in animal models^{4,5}. Less work has been done on TAA induction of immune responses using human antigens and effector cells. Golub *et al.*⁶ demonstrated *in vitro* sensitisation against antigens present on autochthonous Burkitt lymphoma cells. Since such sensitised cells mediate tumour or allograft rejection reactions *in vivo*^{7,8}, the *in vitro* 'education' of lymphocytes against TAA is an appealing

Table 1 Sensitisation of 14L on MLA14 or MLA10

Assay	Effector cells		
	14L	14L+MLA14	14L+MLA10
Average c.p.m. ³ H-thymidine incorporated/10 ⁵ lymphocytes ± s.e.	444±28	864±42	658±25
<i>R</i>	1.00	1.95	1.48
<i>P</i> *	—	<0.0005	<0.005
Average c.p.m. retained MLA14 target cells ± s.e.	3,042±149	1,419±101	2,256±97
<i>C</i> / MLA14 target cells	—	0.53	0.26
<i>P</i>	—	<0.0005	<0.005
Average c.p.m. retained MLA14F target cells ± s.e.	845±27	797±42	860±23
<i>C</i> / MLA14F target cells	—	0.06	-0.02
<i>P</i>	—	n.s.	n.s.
Average c.p.m. retained MLA10 target cells ± s.e.	1,215±76	770±40	769±80
<i>C</i> / MLA10 target cells	—	0.37	0.37
<i>P</i>	—	<0.0005	<0.005

* *P* values were determined by one-tailed Student's *t* test by comparison of control culture (14L) to sensitised culture; ns, not significant at 0.05 level.

but not against fibroblasts. Autochthonous sensitisation led to greater proliferative and cytotoxic responses than did allogeneic sensitisation. This result was not a consistent finding (Table 2) and may be related to the varying antigenicity in the cell lines, because MLA14 has been our strongest stimulator cell.

Four further melanoma sensitisation experiments are summarised in Table 2. Lymphocyte samples from all three melanoma patients were sensitised against the autochthonous tumour line as judged by increased cytotoxic activity. In each case, the increased cytotoxic activity was only detectable against melanoma target cells, but not against tumour cells of different histological types or against autochthonous fibroblasts. The sole exception was some slight 'non specific' cytotoxic activity of the MLA 4-sensitised 4L against the SLA1 target. Thus, the cytotoxic activity generated by sensitisation on autochthonous melanoma cells appeared to be specific for a melanoma-associated antigen. Sensitisation on allogeneic melanoma cells resulted in even greater cytotoxic activity against either the allogeneic or autochthonous tumour target cells in two of the three experiments. Again, no cytotoxic activity against autochthonous fibroblasts was detected. Mitomycin-treated stimulator cells alone were not toxic to target cells, nor did they incorporate ³H-thymidine.

Lymphocyte proliferation after sensitisation on autochthonous tumour cells was much more variable than was cytotoxicity. Only experiments 1 and 3 showed any significant increased incorporation of ³H-thymidine, while none was evident on 10L sensitised on MLA10 (experiments 4 and 5). Autochthonous sensitisation of 10L was less vigorous in cytotoxic response as well. The differences among patients may be related to differences in clinical status or perhaps to the kinetics of lymphocyte proliferation. Preliminary evidence indicates that the two lymphocyte functions, proliferation and

enhancement of cytotoxic activity, may follow different time courses with the peak of proliferative activity preceding the peak of tumour-specific cytotoxic activity. This finding is similar to reports from other investigators¹³⁻¹⁵ stating that the proliferative and cytotoxic phases of alloantigen sensitisation can be dissociated. Details of the time and dose requirements for sensitisation in our system will be reported elsewhere.

The antigens to which the lymphocytes were responding appeared to be melanoma-associated because of the pattern of cytotoxic activity and the requirement for melanoma antigens for sensitisation. Sensitisation on autochthonous normal fibroblasts (Table 2, experiments 6 and 7) did not result in significant cytotoxicity against any target cell. Lymphocytes from patient 4 were sensitised on autochthonous fibroblasts and yielded no significant cytotoxic activity against any target. Similar results have been obtained with 10L on MLA10F and 14L on MLA14F. Thus, the sensitisation procedure as used in these studies appeared to require the presence of the tumour-associated antigens, and was not simply the result of the cocultivation of lymphocytes with another cell type. We are now looking for culture conditions which may allow autosensitisation against normal 'self' antigens expressed on both the fibroblasts and tumour cells¹⁶. Preliminary experiments indicate that sensitisation on allogeneic sarcoma cells does not result in cytotoxicity against the autochthonous melanoma target, again indicating the requirement for the melanoma antigens.

The failure of the lymphocytes sensitised on autochthonous cells to kill autochthonous fibroblasts was an especially important control as both these melanoma cells and fibroblasts expressed HL-A antigens in a quantitatively similar fashion (R. Reisfeld, personal communication). Both cell types were equally, or nearly equally, sensitive to effector cells sensitised against the alloantigens by mixed lymphocyte culture (MLC)

Table 2 Sensitisation of lymphocytes *in vitro*

Experiment no.	Responder lymphocytes (effector cells)	Sensitising cell	Response index	Autochthonous melanoma target	Cytotoxic index		
					Autochthonous fibroblast target	Allogeneic melanoma target ‡	Allogeneic sarcoma or target ‡
2	14L	MLA14	1.28	0.19*	0.06	0.17*	<0.00
3	4L	MLA4	3.37†	0.43†	—	0.43†	0.22*
	4L	MLA10	7.72†	0.49†	—	0.47†	0.12
4	10L	MLA10	0.57	0.22*	<0.00	0.09	0.00
5	10L	MLA10	0.53	0.39*	0.10	0.28*	
	10L	MLA14	3.68*	0.70*	0.00	0.50*	
6	4L	MLA4F	1.84*	0.16	<0.00	<0.00	
7	4L	MLA4F	0.18	<0.00	0.04	<0.00	
8	D	14L	9.19†	0.54† (MLA14)	0.50† (MLA14F)		
9	G	4L	11.57†				
	(150 : 1)			0.46† (MLA4)	0.25† (MLA4F)		
	(50 : 1)			0.21†	0.18†		

* *P* < 0.05 by Student's *t* test when compared with control lymphocytes.

† *P* < 0.005.

‡ Allogeneic melanoma target cells were MLA10 in experiments 2 and 3 and 7, MLA4 in experiment 4, and MLA14 in experiment 5 and 6. Allogeneic target cells of different histological types were BT20 breast carcinoma in experiments 2 and 4, and SLA1 sarcoma in experiment 3.

(experiments 8 and 9, Table 2). In these experiments, lymphocytes from normal donors D or G were sensitised in MLC with mitomycin-treated lymphocytes from patients 4 or 14. The recovered lymphocytes were then tested for cytotoxic activity against both the melanoma cells and the fibroblasts from the donor of the stimulator lymphocytes. It was clear that the fibroblast targets were sensitive to the action of effector cells and were not resistant to lysis.

In summary, it is possible to generate a cytotoxic response *in vitro* against antigens that seem to be tumour-associated. If such sensitised cells have specific cytotoxic activity *in vivo* as well, they may provide a useful therapeutic agent.

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B and T-cell stimulatory activities of multiple mitogens from pokeweed

MITOGENS, which stimulate cell division in immunocompetent cells, are being used in the study of control mechanisms in cellular division and differentiation. In general mitogens are specific for one major class of cells. To stimulate immunocompetent cells^{1,2}, most soluble plant mitogens such as concanavalin A (con A) and phytohaemagglutinin require the presence of thymus-dependent lymphocytes (T cells). On the other hand, bacterial lipopolysaccharide (LPS) stimulates the B (thymus independent) class of lymphocytes³. In contrast, extracts of *Phytolacca americana* (pokeweed) are reported to

have mitogenic activities directed toward both T and B lymphoid cells⁴⁻⁷. A study of the pokeweed system may provide further information on the differences and similarities in the mechanisms by which these two classes of lymphoid cells are stimulated.

We have isolated and characterised five mitogenic proteins from *Phytolacca americana*⁸, designated Pa-1 to Pa-5. They are all mitogenic for murine spleen cells over an unusually wide range of protein concentrations. We now report their ability to stimulate mitosis in different populations of murine lymphoid cells. A complete set of data for Pa-3 is not included in this study because of the very low yield of this protein.

The mitotic responses of the different lymphoid populations were measured by the incorporation of ³H-thymidine into the newly synthesised DNA. The cells (4×10^5 per well) were cultured by the micro method in the presence of the mitogen for 48 h.

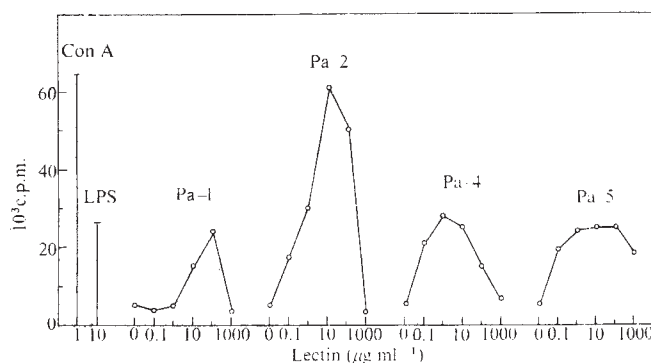


Fig. 1 Incorporation of ³H-thymidine by normal spleen cells from BALB/c mice in the presence of the pokeweed mitogens. Con A ($1 \mu\text{g ml}^{-1}$) and LPS ($10 \mu\text{g ml}^{-1}$) were included as controls for the stimulation of T^{1,2} and B³ cells respectively. Results are the geometric mean of triplicate cultures.

They were then pulsed with $1 \mu\text{Ci}$ of ³H-thymidine and collected 18 h later as previously described⁸.

The dose-response curves of normal spleen cells to these mitogens is presented in Fig. 1. Con A, LPS and the four pokeweed mitogens stimulated this mixed population of T and B cells. The maximum responses of Pa-1, Pa-4 and Pa-5 were about the same and occurred with concentrations between 1 and $100 \mu\text{g ml}^{-1}$. Pa-2 induced a considerably higher incorporation, equivalent to that caused by con A, and its maximum occurred at $10 \mu\text{g ml}^{-1}$. It is unusual for lectins to be mitogenic over such a wide range of concentration. For example, con A is mitogenic over a range of a few $\mu\text{g ml}^{-1}$ (refs 4, 9). In contrast, the B cell mitogen, LPS, is active over a wide range of concentration³.

Figure 2 shows the dose-response curves of thymocytes to these mitogens. Incorporation was less than with spleen cells, but the ratios of the maximum stimulated values to the control values were greater. The response to LPS was the same as the unstimulated control. This is consistent with the absence of B cells and with our observation that less than 1% of thymocytes stain for immunoglobulin when labelled with fluorescent antibody. Con A, the specific T cell mitogen, and all four pokeweed mitogens stimulated these thymocytes. Once again, Pa-1, Pa-4 and Pa-5 showed roughly equal activities, about half that of con A, and produced maximal stimulation between about 10 and $100 \mu\text{g ml}^{-1}$. Pa-2 was the most active mitogen by far, with an activity about three times that of con A. These results clearly indicate that the four pokeweed mitogens have stimulatory activities directed toward T cells.

To evaluate the mitogenic activities of these pokeweed proteins toward B cells, assays were performed using spleens from TXBM (see legend to Fig. 3) mice and spleens from nude mice. The dose response curves of TXBM spleen cells to these

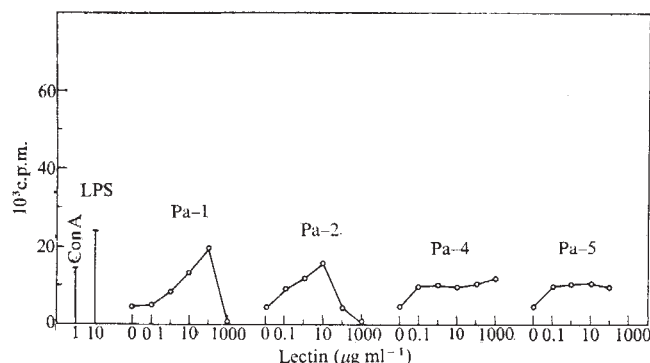


Fig. 2 Incorporation of ^3H -thymidine by normal thymocytes from BALB/c mice. See legend to Fig. 1.

mitogens are presented in Fig. 3. The unstimulated control values were the same as with normal spleen cells (Fig. 1), but there was a marked decrease in the response to con A. The response of TXBM spleen cells to LPS remained at about the same level as with normal spleen cells. Of the four pokeweed mitogens, only the response to Pa-1 remained near the normal spleen cell value. The response to Pa-2 decreased to about a quarter, and the responses to Pa-4 and Pa-5 dropped to about one-half of their values with normal spleen cells (Fig. 1). The diminished but substantial response to con A may indicate the survival of a population of T cells in these TXBM mice as reported by others. The observation that both LPS and Pa-1 were the only mitogens to cause responses similar to those with normal spleen cells suggests that Pa-1 may be mitogenic for B cells as well as T cells. The presence of T cells, indicated by the response to con A, makes it impossible to conclude whether or not the response to Pa-1 and the lower responses to Pa-2, Pa-4 and Pa-5 are due to B cell activities on their part or due to residual T cells in the cultures.

This ambiguity is resolved by the results with spleen cells from athymic (nude) mice (Fig. 4). The unstimulated control values were the same as with normal and TXBM spleen cells (Figs 1 and 3). There was no response, however, to con A, consistent with the absence of T cells, and the B cell response to LPS was increased. Of the four pokeweed mitogens, only Pa-1 stimulated these B cells.

All these results suggest that in the murine system Pa-1 is mitogenic for both T and B classes of lymphoid cells and that Pa-2, Pa-4 and Pa-5 are mitogenic for only the T class. While the trace mitogen, Pa-3, was not included in all these experiments our preliminary results indicate that it has biological properties similar to Pa-2 but is less potent.

The differences in the mitogenic specificities of these proteins

are directly reflected in their physicochemical properties⁸. Pa-1 differs markedly in these properties from the four other pokeweed mitogens. Pa-1 has a subunit molecular weight of 22,000, but behaves as a multimer or aggregate during gel filtration in non-dissociating solvents. Pa-1 also differs noticeably from the other four proteins in its amino acid composition and has few disulphide bonds. In contrast, Pa-2, Pa-3, Pa-4 and Pa-5 are similar to one another in amino acid composition, have many disulphide bonds, and appear to be monomers⁸.

The polymeric state of Pa-1 may be the key to its ability to stimulate B cells. It may be that B cell activation requires the interaction of several cell receptors with multimeric stimulants. This concept is in accord with the finding that attachment of T cell mitogens such as con A to the bottom of a Petri dish¹⁰ or Agarose beads⁶ induces B cell activity. Indeed, the attachment of the pokeweed T cell mitogens Pa-2, Pa-4 and Pa-5 to beads

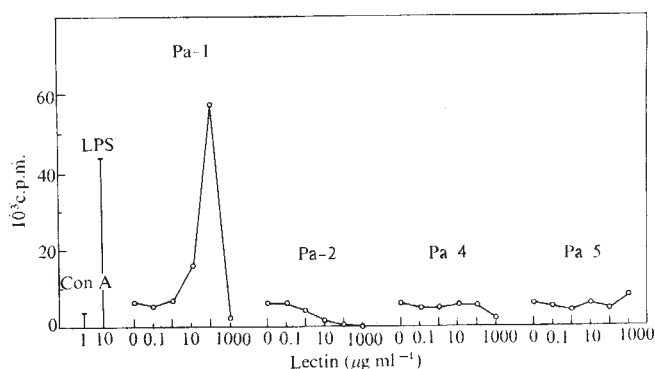


Fig. 4 Incorporation of ^3H -thymidine by nude spleen cells obtained from athymic mice bearing the *nu/nu* gene on a Swiss genetic background. The responses of normal littermates did not differ significantly from the BALB/c spleen cells. See legend to Fig. 1.

induces B cell activity (our unpublished results). The requirement of multiple interactions of the B cell immunoglobulin receptors with antigens¹¹ and antibodies^{12,13} for stimulation has been reported and discussed. In a similar way, the multimeric state of Pa-1 and the spatial complex of repeating mitogen molecules attached to beads may react with the B cell to induce mitosis.

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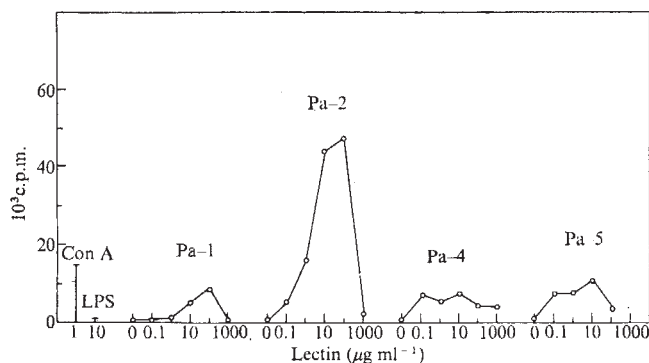


Fig. 3 Incorporation of ^3H -thymidine by TXBM spleen cells. BALB/c mice (4–6 weeks old) were thymectomised, 10 d later they were X irradiated (900 rad) and reconstituted with 7×10^6 anti- θ -treated bone marrow cells from littermates. The spleens were used 4–6 weeks later. See legend to Fig. 1.

matters arising

Liquid drop model of elastin

WE wish to show how the calorimetric observations of Weis-Fogh and Andersen¹ may be interpreted in terms of the classical theory of rubber elasticity. It was observed by Weis-Fogh and Andersen that when, for example, 1.33 mcalorie of work was done on a specimen of elastin immersed in water at room temperature, the heat evolved was 5.53 mcalorie: according to the theory of rubber elasticity the heat evolved should be close to 1.33 mcalorie. This experiment and others of a similar nature with water-ethanol solutions as solvent lead Weis-Fogh and Andersen to reject the classical theory² which they replaced by a two-phase model, the liquid-drop elastomer.

In approaching this anomaly we formulated the working hypothesis that the apparent conflict between the theory of rubber elasticity and the calorimetric experiments was due to the erroneous assumption that heat change due to the stress-induced absorption of solvent was negligible. If a specimen is immersed in a permeating solvent and elongated then it is to be expected that solvent will diffuse into the specimen so that the heat liberated will depend on the magnitude of the differential heat of dilution, $\overline{\Delta H}$ (ref. 3).

The most striking observation of Weis-Fogh and Andersen¹ is the systematic decrease in heat release for the elastin-water/ethanol system as the ethanol content of the solvent increases from 0 to 50%. At 20% ethanol the heat release equals the work done, in agreement with the theory of rubber elasticity. This can be explained in one way only, if our working hypothesis be correct. At 20% ethanol $\overline{\Delta H}$ must be zero: below 20% ethanol $\overline{\Delta H}$ must be negative and above 20% ethanol it must be positive.

In order to determine the magnitude of $\overline{\Delta H}$ it is necessary to determine three partial derivatives³,

$$\overline{\Delta H} = -T \frac{(\partial f / \partial N)_{PLT} (\partial N / \partial T)_{PLc}}{(\partial N / \partial L)_{PTc}} \quad (1)$$

in which f is force, P pressure, L length, T temperature and e signifies that the specimen is in equilibrium with a reservoir of diluent from which it has absorbed N mol. Of the three partial derivatives only one, $(\partial N / \partial T)_{PLc}$ can be measured easily. Both $(\partial f / \partial N)_{PLT}$ and $(\partial N / \partial L)_{PTc}$ can be measured but with greater difficulty. Since we required only the sign of $\overline{\Delta H}$ we measured $(\partial N / \partial T)_{PLc}$ and assumed

$(\partial f / \partial N)_{PLT}$ to be negative and $(\partial N / \partial L)_{PTc}$ positive. This assumption is based on sound *a priori* arguments (the opposite assumption would be extremely rash) and is in accord with observation; for instance Abe and Prins³ for the system water-polyvinyl alcohol. It follows then that $\overline{\Delta H}$ will have the same sign as $(\partial N / \partial T)_{PLc}$.

Measurements of $(\partial N / \partial T)_{PLc}$ on purified pig aorta at temperatures between 0° and 50° C, have shown that at 20% ethanol $(\partial N / \partial T)_{PLc} = 0$. Below 20% ethanol $(\partial N / \partial T)_{PLc}$ is negative and above it is positive. Thus $\overline{\Delta H} = 0$ at 20% ethanol and at this composition the system behaves as a classical elastomer in the calorimetric experiments. Full details of this work will be published in due course.

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The Gibbs Fracture Zone

I WISH to comment on the idea that the Gibbs Fracture Zone may be a westward projection of the Hercynian front into North America. Cherkis *et al.*¹ have argued in favour of a correlation between the Gibbs Fracture Zone and the late Palaeozoic Hercynian orogenic front.

Geophysical evidence² indicates that the Gibbs Fracture Zone extends from the Newfoundland continental margin near 52°N 47.5°W to a point west of Ireland, near 52.25°N 16°W. Off Newfoundland the fracture zone seems to be associated with a 200 km sinistral offset of the continental edge³.

If Wilson's theory for the propagation of oceanic fracture zones from old lines of weakness in a formerly contiguous continental crust is applicable here, then an equivalent offset should be present in the European margin. Such an offset has not, as yet, been found. It is clear that one does not exist in the western margin of Porcupine Bank as implied by the argument of Cherkis *et al.*¹. A possible site is near

the mouth of Rockall Trough, but the shape of the continent-ocean boundary in this region is not well known.

Despite this lack of knowledge about the eastern end of the Gibbs Fracture Zone, it is instructive to follow Wilson's theory and see which pre-drift continental lineament aligns best with it. To do this, I have drawn Palaeozoic structural features of Newfoundland and the British Isles on a new reconstruction of the North Atlantic continents (Fig. 1). This reconstruction is more consistent with current knowledge of the structure of the Atlantic Ocean basin than is the reconstruction of Bullard *et al.*⁵ which was apparently used by Cherkis *et al.*, after Hurley. The trends of the structures in Ireland are taken from Kennedy *et al.*⁶. The westward projection of the Hercynian front has not, to my knowledge, been defined by geophysical work, but in the Celtic Sea to the south, a west-south-west Hercynian trend may exist⁷. It is therefore possible that the front trends west-south-west. In the case of the Caledonoid lineaments there is some

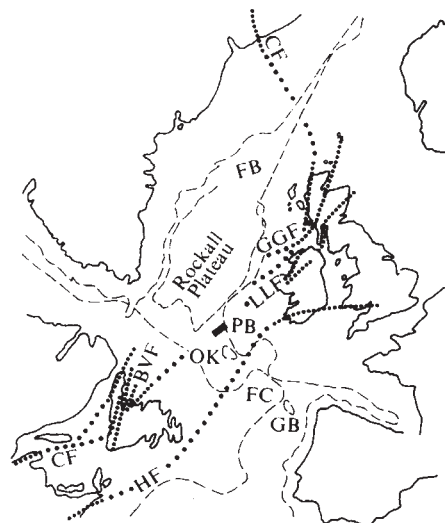


Fig. 1 A reconstruction of the North Atlantic continents (from Scrutton, R. A., unpublished) showing Palaeozoic structural lineaments. Closely dotted lines, well known trends; widely dotted lines, less well known or conjectural; solid rectangle, the position in which the Gibbs Fracture Zone developed during North Atlantic opening; FB, Faeroes Bank; PB, Porcupine Bank; OK, Orphan Knoll; FC, Flemish Cap; GB, Galicia Bank; CF, Caledonian Front; HF, Hercynian Front; GGF, Great Glen Fault system; LLF, Leck-Leannan Fault; BVF, Baie Verte Fold.

magnetic evidence for a general westerly projection towards the outer edge of Porcupine Bank⁸. Clearly, correlation of the Gibbs Fracture Zone with any of these structural trends, in particular the Hercynian front, is tenuous.

Trends in and around Newfoundland are taken from refs 3 and 6. At present, there is no evidence from Newfoundland of any thrust that can be considered as the equivalent of the Hercynian thrust front of New Brunswick and the British Isles. The magnetic anomaly lineations, which Cherkis *et al.* suggest indicate the Hercynian Front, in fact overlie a zone of mafic and ultramafic intrusions of Caledonian age⁹ and parallel the Caledonoid Hermitage Flexure. The Hercynian Front is therefore taken south of Newfoundland to meet the continental edge between Orphan Knoll and Flemish Cap. The Caledonian structural trends apparently continue north-eastwards from Newfoundland to the continental edge near the Gibbs Fracture Zone³; it could be one of these rather than the Hercynian Front that best correlates with the fracture zone.

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REPLY: Scrutton's argument that no offset has been found to link the Gibbs Fracture Zone to the European continent is certainly valid. Likewise, his proposed site for the existence of the offset "near the mouth of Rockall Trough" deserves attention. We did not, however, suggest¹ that the Gibbs Fracture Zone is represented by the Hercynian Front, but rather that the Hercynian Front provided the zone of weakness in the Proto-Atlantic Ocean, along which a transform fault evolved on the Mid-Atlantic Ridge after the Atlantic [re]opened.

Using seismic profiling we have determined that the Gibbs Fracture Zone can be traced to 16°W. At that point, the relative proximity of the Porcupine Bank means that the fracture zone cannot turn northward into Rockall Trough. The lineation trend of the Hercynian Front in southern Ireland cannot be ignored. Projecting this trend across the Porcupine Bank from the Irish coast to the Gibbs Fracture Zone (admittedly, a 180-mile gap) the match is too perfect to be mere coincidence. Gray and Stacey² found gravity complexities over the Porcupine Bank and Bight that show that positive free-air anomalies terminate abruptly along the projected frontal line. Vogt and Avery (Fig. 16 of ref. 3) showed that negative magnetic anomalies occur along the projected frontal line, interrupting the north-south lineations.

Regarding the North American link, Grant showed the Gibbs Fracture Zone coming into the continental margin, and crossing a fault plane. We projected the fracture zone through the fault plane and through central Newfoundland, *en echelon* with, or coinciding with, the Caledonian Front. An alternative suggestion is that the fault plane may include a strike-slip line, offsetting the Gibbs Fracture Zone to the south through Flemish Pass, and then continuing its easterly course through Nova Scotia and New Brunswick. Unfortunately, magnetic measurements do not confirm this; they indicate a more northerly pattern than Scrutton implies from his Fig. 1, that is, across Cabot Strait, rather than toward Chedabucto Bay and Nova Scotia. Furthermore, Rast and Grant's³ observations of Hercynian structures in New Brunswick seem to confirm the magnetic trend.

The answers to these questions can be solved only with more detailed geophysical studies of the areas.

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External origin for lunar rilled craters and the mare basins

FLAT-FLOORED craters on the periphery of the lunar maria often contain rilles. Because of their location, Whitford-Stark¹ has proposed that these craters are of internal origin and that the mare basins also must be so. The

assumption that "rilles in all internally rilled craters formed at about the same time as the crater floor" is central to Whitford-Stark's theory. Yet, Repsold, Vasco da Gama R., and other ancient, broken craters contain sharp rilles, which often cut mare albedo floors. The presence of mare material (lava) on the floors of ancient pre-Imbrian craters shows that the floors, and therefore the rilles, formed after the craters.

Quantitative support for partial flooding of rilled craters by lava comes from Whitford-Stark's depth-diameter diagram which illustrates that the majority of rilled craters are shallower than normal 'eumorphic' craters. Additional evidence of lava fill is provided by his observation that the floors of rilled craters generally have a lower crater density than the floors of non-rilled craters of the same stratigraphical age. Thus, the difference between rilled and non-rilled craters need not result from different origins, but rather, may depend upon whether they have been flooded by lava. Similarly, the wide scatter of depths of craters of Imbrian age may have resulted from variable depths of lava fill. In this context it may be noteworthy that nearly 50% of the craters plotted on Whitford-Stark's depth-diameter diagram are of Imbrian age; that is to say that the craters were formed when magma was abundantly available.

There is an alternative to the hypothesis of internal origin for rilled craters. Mare basins resulting from gigantic impacts² profoundly fractured the lunar crust, creating conduits to the surface for magmas which were extruded approximately 200 to 800 Myr later³. Craters, predominantly of impact origin (ref. 4, and Wood, C. A., unpublished), that formed near the basins were often flooded by magma rising up basin fractures. Rilles could then form, as Whitford-Stark suggested, by contraction of cooling lava ponded within the craters, or by the fracturing of tholoid domes. Rilled craters could also form along other zones of structural weakness—such as the Rheita valley¹—which would provide fractures for magma ascent. Thus, although the formation of rilles within craters is a volcanic process, the craters themselves, and the basin fractures, most likely result from impact.

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reviews

Neurones or semiconductors?

On Machine Intelligence. By Donald Michie. Pp. xi+199. (Edinburgh University : Edinburgh, April 1974.) £2.50.

THIS book is a collection of "semi-popular essays written over the past ten years or so". The author's criterion for inclusion is stated with candour: "If I enjoyed writing the essay in the first place, and if now I enjoy re-reading it, then I have put it in, otherwise not". The book contains a great deal of repetition—the same tables appear in several different articles, and the same themes occur over and over again with little variation in their treatment. Some articles even contain quotations from others.

Since the essays were originally intended to be popular, the only work that is described in detail is of a very simple nature such as the author's device for learning to play a good game of noughts and crosses by trial and error. Games playing programs receive adequate coverage but there is little attempt to describe the principles behind more recent programs designed to analyse scenes or to understand natural language. Readers are told that "powerful new programming aids, such as the PLANNER, QA 4, and CONNIVER languages have come into play", but they are not told what it is about these languages that makes them an advance on previous information processing languages. The author has considerable expository skills, and within the limits he sets himself the writing is lively and clear. It is a great pity that he has not sat down to write from scratch a systematic introduction to artificial intelligence for the general scientific reader: such a book is badly needed and there are few people as well qualified to write it as Donald Michie.

The book makes two major claims. Michie asserts that developments in artificial intelligence, or as he prefers to call it machine intelligence, will bring about a technological revolution. According to "experts", robot chauffeurs and computer psychiatrists will be commercially available by the year 2000 though it will take a further 10 years before automated "general factotums" come off the automated assembly line. It may or may not be technically feasible to produce machines that can make as intelligent a use of a wide range of

knowledge as do chauffeurs and psychiatrists. Leaving this problem aside, there are two arguments, not considered by Michie, which suggest that machine intelligence may not have such a wide role in human affairs by the end of the century.

First, in automating a given system, it is often best to redesign it so as to obviate the need for any application of intelligence. Automatic pilots do not function in an intelligent way: they land planes by flying down a radar beam, and this slavish procedure circumvents the complex perceptual processes used by human pilots. It will probably be cheaper and safer to redesign road transport systems in this sort of way, rather than to provide robot chauffeurs that will have to make the same kind of intelligent decisions as a human driver.

Second, where automation by the use of unintelligent machines cannot be achieved, as presumably in the case of the psychiatrist, human intelligence is likely to remain for a long time to come a cheaper method of doing the job than the use of large computing systems. I once estimated that the cost of a neurone in the brain of a young professionally qualified adult was about 0.00004 new pence. This estimate includes the programming costs for the whole system and it is orders of magnitude cheaper than the cost of a Japanese semiconductor. The extent to which artificial intelligence makes a contribution to technology depends on cost-effectiveness, and even if the problems of making intelligent systems were solved, it is by no means obvious that they would be the cheapest means of undertaking tasks requiring intelligence.

Michie's second claim is that machine intelligence is likely to provide new insights into the working of the human mind, and this assertion is perhaps less disputable. Until recently, psychologists did not have a language in which they could couch well formulated theories of how complex information processing tasks are carried out. Computer languages provide the appropriate symbolism and the digital computer itself provides a method of rigorously exploring the consequences of a given theory. It is perhaps unlikely, however, that programs that simulate the details of the way the brain operates will be

written merely by reflecting on the best way in which to program a computer to undertake a particular task. It will be necessary also to take into account the experimental evidence on how the brain actually does process information. Michie (page 123) asks his readers whether, when parsing a grammatically ambiguous sentence, "you generate the alternative parsings, subsequently to be eliminated by semantic hindsight. Of course you don't. We can be sure that we do not do this consciously". But recent psychological evidence indicates that at least in some cases we do generate different interpretations at an unconscious level. Only one of these may be selected as a result of semantic disambiguation, and it is only the results of such selection that appear in consciousness. The study of the mind from an armchair or a programmer's stool contains dangers of this sort, and it is likely that our understanding will be best forwarded by using the computer as a tool with which to construct and to explore theories, at the same time being careful to base the theories on the best experimental evidence available.

N. S. SUTHERLAND

Teaching the heart

Universities Between Two Worlds. By Roy W. Niblett. Pp. viii+179. (University of London: London, June 1974.) £2.85.

ARE we, in the Western world, at a watershed of civilisation, "between two worlds"? Professor Niblett believes that we are, and in this book he spells out what he regards as some of the consequences for universities. His theme is familiar: the growing disenchantment with the emphasis which universities put upon the cognitive, the rational, the analytical approach to phenomena; the insufficiency of this approach to solve the problems of contemporary society; the need, therefore, for universities to provide experience in "sensate culture", to ask questions about ends as well as means, about values as well as facts, to engage (as Niblett puts it) the "individual mind and heart" in the process of higher education. It is a plea to university teachers to impart not just knowledge, but wisdom, to their pupils.

That Western man is deficient in wisdom and has, if anything, a surfeit of knowledge (much of it useless) is a proposition few would challenge. But

that universities are the institutions to remedy this deficiency is another matter. Niblett believes that they are, and the value of this thoughtful essay is that the reader must decide whether he accepts Niblett's belief or not.

His diagnosis of higher education (he has in mind particularly Britain and America) is that it has tried to break away from the pursuit of narrow specialisms, and has failed. Liberal studies, joint honours schools, the redrawn 'map of learning' in the new universities, the Harvard red book on general education: all have led to disappointment. The causes of disappointment include the inflexibility of discipline-oriented teachers, the persistence of competition in examinations, the lure of professionalism, and pedantry among those who teach the humanities. "The choice before the humanities is clear: either to pretend to be sciences or to acknowledge without shame the experiencing and imaginative elements within any essential study of them." The word 'feelings' occurs often throughout the book; feelings must be recognised, identified, and incorporated into the academic experience.

In his courteous and gentle way, Niblett makes some highly controversial assumptions which some of his readers will challenge. First, are we really between two worlds? Is this a watershed similar to the great divide between Galileo and the Schoolmen? Many will question this assumption. The scientific approach to nature and to man still succeeds superbly in answering scientific questions; it was never designed to answer other sorts of questions. Nor are the other sorts of questions novel, needing new techniques. As Niblett himself suggests, you do not "decide to love or trust another human being"; so case studies in decision making (a popular form of broader education) do not embrace some of the essential human activities. But societies have been concerned for millennia with love, trust, and the whole spectrum of moral values.

The burden of Niblett's message is precisely the same as Walter Moberly's, written 25 years ago, and it is a response to the same dilemma. The only difference is that in 1949 only dons appreciated the dilemma; today students appreciate it too. The dilemma is that universities, in common with madrasas in the muslim world and the ancient Buddhist seminaries of India, have for six sevenths of their history been religious institutions, resting on a foundation of faith, commanding a consensus of values. It is only since University College, London, was founded that the formal link between faith, morals, and learning was broken. Both Niblett and Moberly believe (though they approach the issue from

different directions) that universities should adapt themselves to the consequences of this severance. But some readers will ask: should they? There surely is a case for differentiation of functions among institutions. When the church tried to meddle with science in the 19th century, it was religion, and the sensate culture associated with it which suffered. If the universities now try overtly to meddle in the non-cognitive sphere, important as it undoubtedly is, is there not a risk that they will prove incompetent (professors are no longer elected on grounds of virtue or wisdom) and that the university's unique function, to teach the art of rational analysis, will be eroded? If the purpose of the book is to stimulate reflections of this kind, it deserves to be widely read.

Niblett himself admits that there is no clear way to teach the sensate culture. And indeed some of us who are close to students doubt whether they are as impoverished in non-cognitive experience as he supposes. Not enough of them find, as he puts it, "a sense of values in an age of facts". This has not been my experience. I find more concern with moral issues, more compassion, and a livelier (if puzzling) commitment to the sensate culture among today's students than among those who were students when Niblett and I were undergraduates. I wonder whether (perhaps) they are getting on quite well, thank you, without our help. It is a humiliating admission for an academic to make after a lifetime in universities; but I, for one, would admit that despite all our anxious design of curricula and the student environment, most students get from universities not what we plan to give them but what they come to find. Over their morals and personal values we cannot do in three years what home, school, and church may have left undone in the previous 18 years. Over their adolescent intellects we can do a lot: we can open the doors of the mind.

ERIC ASHBY

Extended scope of NMR

NMR of Paramagnetic Molecules: Principles and Applications. Edited by G. N. La Mar, W. DeW. Horrocks, jun., and R. H. Holm. Pp. xv+678. (Academic: New York and London, December 1973.) \$42; £19.25.

ALTHOUGH the effects of small amounts of paramagnetic impurities on nuclear resonances of diamagnetic solid or liquid samples were discussed in some of the earliest work on NMR in bulk matter, it was not until 1956 that direct observations on the paramagnetic crystal, MnF_2 , by Shulman and Jacarino were described and interpreted in terms of chemical bonding. Since

then it has been realised that although the powerful electronic magnetic moments in paramagnetic substances induce strong nuclear relaxation in neighbouring atoms in the same compound, the effect is often none the less weak enough to allow a great deal of the information in the nuclear resonance spectrum to be obtained. The modulation of the nuclear resonance by the paramagnetic moment turns out to be of the greatest importance, and measurements of NMR spectra of paramagnetic substances have extended in a most dramatic way the application of NMR to such diverse fields on the physical chemistry of transition metal complexes and the structures of enzymes.

Professors La Mar, Horrocks and Holm have succeeded in persuading an authoritative group of authors to set out the theoretical bases of this important area of research and to review the range of applications which have been explored up to 1973.

The first three chapters by Jesson, Swift and by La Mar, comprising about one fifth of the book, give an excellent account of the basic theory and are so clear and well set out that they will be of great value to the beginner who already has an understanding of the principles of magnetic resonance, as well as to the researcher needing a convenient summary. The chapter by Jesson sets out the theory of the paramagnetic shift, including a very clear account of the Kurland and McGarvey general treatment. This is illustrated by some examples and at the end of the chapter there is an appendix on density matrix theory. The second chapter by Swift deals with the effect of the paramagnetic moment on relaxation times. This is a very good and concise exposition of the various effects needed to interpret the spectra. In the third chapter the theory of the delocalisation of spin density is set out in detail.

The remaining thirteen chapters review the applications of these principles to an extraordinary range of chemical studies including spin distribution over organic and organometallic compounds, stereochemistry, kinetics of intramolecular rearrangements, equilibria in solution, and applications to biochemical studies. These review chapters present illustrative examples which are worked out in some detail, for an understanding of which a careful study of the first three chapters of the book is a good preparation.

This is a most valuable book for the many research workers who could make good use of measurements of this kind, though the sheer range and versatility of the problems described will be daunting to a beginner.

R. E. RICHARDS

Peripatetic naturalist

Linnaeus's Öland and Gotland Journey 1741. Translated from the Swedish edition of 1745 by Marie Åsberg and William T. Stearn. Pp. viii+220. (Academic: London; Linnean Society of London, February 1974.) £6; \$17.50.

IN 1741, at the age of thirty-four, Carl Linnaeus was already an eminent naturalist and professor of medicine when he set out to survey the natural resources of the Baltic islands of Öland and Gotland. Taking with him six male companions, he travelled at the behest of the Swedish Parliament, which was intent on economic recovery through the exploitation of domestic raw materials. While familiarising himself with the natural history of the islands, Linnaeus was instructed particularly to look for plants suitable for making dyes, or with pharmacognostic value, and for clay or earth suitable for the production of such things as pottery and pipes. He was also required to keep an accurate diary, which was published in 1745 and is now made accessible to English speaking readers by Drs Åsberg and Stearn in their enjoyable translation. The style is that of daily field notes that have been tidied up grammatically, but not turned into a continuous narrative. Although not competing as bedtime reading with the picaresque novels of the period, the diary provides some interesting insights into the observational methods and common sense attitudes of the man who is remembered chiefly for giving the world the binomial system of nomenclature for plants and animals.

The members of the expedition left Stockholm on May 15, and, travelling on horseback, reached the coastal town of Kalmar on May 27. After waiting in vain for the weather to improve, they embarked on the ferry for the three-mile passage to Öland, hastened by two cases of spotted fever in the house where they had stayed. By July 27, when they returned to the mainland, they had traversed much of this narrow island, as well as Gotland, on which they spent thirty-four days. On the latter, the diary disdainfully records, there were no persons of standing except for two judges and a captain.

Linnaeus was well pleased with the journey. He recorded a hundred plants then unknown in Sweden, including several with pharmaceutical uses, such as *Ruta muraria* (now known as *Asplenium ruta-muraria*) which previously had been imported.

Finding woad (*Isatis*) growing in abundance on Klasen, a small island near Gotland, he recommended that it would be worthwhile to sow the species more extensively. In other places the extraction of dye from plants was recorded assiduously for the parliament.

Usually leaves or roots were boiled together with yarn, which was subsequently treated with an alkaline concoction to fasten the colour.

By such a procedure, yellow dye was obtained from burmarigold (*Bidens tripartita*) and juniper moss (*Cetraria juniperina*), brownish red from *Origanum vulgare*, a lemon colour from apple bark and dark brown from soot. But no use had been found for the red colour of the rotting seaweed that covered some of the shores of Öland, and "gave off such a stench, as if ten horses had been roasted in the sun". Seaweed, however, was put to good use in other places, as manure and for thatching roofs.

Local remedies were found for all manner of ills. These included the moss *Sphagnum palustre* which was cooked in beer and laid on swollen legs; grasshoppers that spat out a black liquid, causing warts to disappear; the root of *Valeriana officinalis* which was used for hysteria (Linnaeus failed to record how), and *Solanum officinarum*, ground up with spiders web and rancid pork to treat whitlows.

While the expedition was at Forsönd on Gotland the farmers left their fields and fences to indulge in feasting and dancing. Here Linnaeus drew a picture of a seamless bagpipe made from a seal's stomach, with the modulator placed in the pylorus and the base inserted into the oesophagus. Seals (*Phoca vitulina*) served other purposes in the economy of Gotland and there are several accounts of hunting methods. The beasts were caught in nets and either harpooned or bludgeoned on their very sensitive noses. Seal lard was sold, the meat was eaten, and the fresh fat could be used instead of butter for frying pancakes. Porpoises were sometimes caught in the nets accidentally, and Linnaeus thought it quite remarkable that nobody had developed the art of trapping them, for their lard was as useful as that from seals.

Interspersed among meticulous accounts of this sort, and descriptions of fences, corn fields, runic stones, flora and fauna, Linnaeus provided notes on local superstitions. This he did "more to entertain my reader than for any other reason". On mainland Sweden the rational professor was not impressed by the belief that when a farmer's cow died he could transfer the bad luck to his neighbour's cattle by burying the carcass in the neighbour's field or dung hill. Such beliefs "and infinitely much more of similar rubbish" were dismissed as relics of heathen and popish times. Linnaeus' prescription for the eradication of such things was to instruct future theologians well in natural history and natural philosophy, since nothing

served so well to check superstitions as the contempt of the clergy. He resolved to teach his students at Uppsala University everything necessary for them to go into the countryside and instruct their parishioners to best advantage. This he did, and much more besides, as botanists know. Now this diary shows them an aspect of Linnaeus with which they may not have been familiar.

MARY LINDLEY

Scientists in the world

Towards a Political Sociology of Science. By Stuart S. Blume. Pp. xii+288. (Free Press: New York; Collier-Macmillan: London, April 1974.) £4.95.

LIKE all really good empirical studies in the human sciences, this one is addressed to a theoretical problem. That is, the nature of the scientific community. The author argues that, for sociological analysis, it must be considered as both involved in national political life and also internally differentiated by power relations between élites and others.

In the post-Rothschild epoch of fully industrialised science this thesis is hardly likely to cause outrage among working scientists. But it does represent an important development in the sociology of science. Lest natural scientists dismiss it on that ground they should recall how naive and short-sighted were the dominant assumptions of scientists' own common sense about the social activity of science all through the postwar period.

In the sociology of science, debate is still organised around the ideas of Robert K. Merton. We can distinguish three phases of 'Mertonism', a set of received research paradigms much simpler than the contemporary enquiries by Merton himself. At the beginning was the attempt, through the 'four norms' and the 'ethos' of science, to identify the implicit value system of scholarship with that of the most enlightened democracy, political and economic. In the post-war period Mertonism shifted to the motivational machinery by which men in this idealised scientific community are induced to merge self interest with the common good. Recently, more realistic details have been introduced, exhibiting the community as a geronto-aristocracy, and seeing how well this social structure works.

Even this 'late Mertonism' imagines a community of 'pure' science, doing its own praiseworthy thing in a social vacuum. Blume contends that such a model is drastically over-simple. His main thesis is that the élites in science are maintained in power partly by their external connections and that they

choose their recruits partly by criteria other than scientific merit. It is then an entirely open question, left as such by Blume, whether this arrangement is the best possible. The reader may, if he wishes, draw the further conclusion that the government of science is ripe for political reform.

It would be misleading to represent the book as structured tightly around this particular polemical thesis. Rather, there is a broad survey, extending into such unaccustomed fields as unionisation and 'politicisation', but everywhere considering science as one institution among many, whose functioning involves 'politics' in every sense of the term. Even where Blume deals with topics traditionally considered as 'internal' to the scientific community, as organisations and 'social control', he unromantically invokes relations of status, authority and power as the basic explanatory principles. The material on "scientists and government" presents few methodological problems.

Oddly enough the concluding chapter on "innovation and society" rings slightly off-true. The case study here, fluoridisation, is of great systematic interest for the sociology of knowledge and control; but it is somewhat outside the main trend of the book, if nothing else than because the 'science' involved is medical (which remains peculiarly remote, intellectually and socially, from the central physical sciences). It may be that in spite of his sympathetic treatment of radical science groups, Blume has missed one crucial feature of the present connection between science and the lay public. This is the major part played by non-scientists, notably critical journalists, who muckrake establishment technology from the social and environmental points of view. Even if their excesses of zeal and haste do occasionally produce errors of fact and method even greater than those of their technically qualified opponents, they are now a permanent part of the scientific scene. Their influence on present recruits to science is not to be overlooked.

Considering this book as a contribution within the sociology of science, we may see it as opening a new phase, a final liberation from the Mertonian paradigm. Being empirical political sociology, it inevitably changes the terms of the debate away from models of a 'scientific community' towards an examination of a quasi-profession that depends on the state and industry for its existence. Sociologists of science no longer need to be tough-minded in demythologising 'science'; the simple facts of life about this sector of production render myths, models and ethos equally irrelevant.

It will be very interesting to see whether there is an effective reaction

against this tendency. If not, we can mark one of the most important changes in the political sociology of science viewed historically. For the ideology of science which (*pace* Blume) has a significant history extending back a couple of centuries, is built into beliefs and practices of many other spheres of activity; the term 'social science' can indicate this. An impairment of the functions performed by that ideology, within science as well as externally, will produce new phenomena quite as interesting as any studied in this present excellent survey.

J. R. RAVETZ

First map of the Moon

Thomas Harriot: Renaissance Scientist. Edited by John W. Shirley. Pp. x+181+4 plates. (Clarendon: Oxford; Oxford University: London, June 1974.) £6.50.

THIS book consists of seven varied essays, which are based on papers read at a symposium held at the University of Delaware on 5-7 April 1971. The editor explains in his preface why we should take an interest in Thomas Harriot (1560-1621): "Though recognised by his contemporaries as England's most profound mathematician, most imaginative and methodical experimental scientist, and first of all Englishmen to make a telescope and turn it on the heavens, Harriot had not prepared his work for future generations". This neglect of self-advertisement was doubtless produced partly by the superstitions of the age that could pillory an astronomer, and partly by the need to keep as state secrets the new techniques in instrumental navigation, the means by which England might survive economically. He did publish, in 1588, his account of a trip with Raleigh to Virginia, and is consequently now known among historians on both sides of the Atlantic as a pioneer, natural historian, ethnographer, and as one who made a systematic study of the Algonquian language. But, as a scientist, he is still the Cheshire Cat's grin.

It becomes apparent that Harriot knew the sine law of refraction before Snel and Descartes, and was the first man to draw a Moon map with the aid of a telescope. His earliest known telescopic observation of the Moon, on July 26, 1609 (OS) at 9 p.m., was made nearly three weeks before Galileo presented his telescope to the Venetian Senate, of which instrument Harriot could have had no knowledge whatever. Again, he may be given priority in the telescopic observation of sunspots, first recorded on December 8, 1610.

The trouble comes when one assesses the significance of Harriot in the history of science. As his pupil and friend Sir William Lower wrote on February 6 1610: "yet too great reservedness hath robbed you of those glories . . . it is possible by too much procrastination to be prevented in the honour of some of your rarest inventions and speculations. Let your country and friends enjoy the comforts they would have in the true and great honour you would purchase yourself by publishing some of your choice works". In this connection, Dr North, in chapter 7, reminds us of Arago's "règle aux historiens des sciences", which was public utility, implying prompt publication. There is no doubt, however, that Harriot contributed to new techniques in navigation and in the training of navigators.

The present volume contains: Professor Rosen on the intellectual background; Professor Shirley on Harriot's service with Raleigh; Professor Quinn on the Virginian expedition; Dr Pepper's highly technical paper on mathematical navigation; Dr Tanner on associates of Harriot, with a gratuitous attack on Henry Stevens (1819-86) who found Harriot's will; Professor Jacquot on the new natural philosophy; Dr North on Harriot's ten telescopes and the earliest observations made with them. These papers show the signs of their symposium origin. The same quotation may appear in different essays, and the references at the ends of the chapters (difficult of access here, why not have them in the proper place at the foot of the page?) and modes of citation are not made completely uniform. The nine-page bibliography lists the primary and secondary manuscripts, books published around Harriot's time, and modern studies relating directly or tenuously to Harriot. The Clarendon Press should have been more careful over consistency in the references (*ibid.* is in italic or roman; journal volume numbers are in arabic or roman), in ensuring that the "selective" index always led to the correct page, that the plate captions were properly positioned, and that type had not slipped.

As Professor Shirley explains, the study of the contemporary evidence, household accounts of the Northumberland, the public records, the Harriot manuscripts, has not been completed. This book of essays is, then, an expensive trailer for the main feature still to be shot. One can query the value of publishing this collection in book form, thus giving it permanence. What is needed first is a detailed description and assessment of the Harriot manuscript material. Only when this was been done can his significance be evaluated.

G. L'E. TURNER

Detecting nuclear particles

Nuclear Electronics. By P. W. Nicholson. Pp. xiv+388. (Wiley: London and New York, April 1974.) £8.75.

THIS book is aimed specifically at physics graduates needing to use nuclear detectors. The first two chapters are introductory accounts of the principles involved in detecting nuclear particles and of the basic ideas of pulse circuits; these chapters are provided on the assumption, which unfortunately is probably correct, that most undergraduate courses contain very little on these topics. The next three chapters give an account of the general problem of the amplification of nuclear pulses and the methods of minimising the effect of noise. This is the most attractive part of the book as the author gives particularly clear explanations of the problems which arise and the methods used to solve them. The remaining chapters deal with discriminators, digitising techniques, for both amplitudes and time differences, and various more specialised circuits. In these sections it is inevitably more difficult to provide any connected chain of argument but the treatment is still reasonably uniform: any theory is given first, the various difficulties and their solution are clearly explained and a well chosen selection of recently published circuits is given as illustration.

This is essentially a text book rather than a practical guide. Brief accounts are included of the operation of some semiconductor devices, but the whole subject of component characteristics and limitations is omitted and there is hardly any mention of practical 'engineering' problems such as interference, effects of stray capacitances or long-term stability.

I think the author has been very successful in carrying out his purpose but unfortunately a detailed theoretical understanding is not sufficient for the experimental physicist, whose problems in practice are of two sorts. First, given a large budget, he has to ask whether commercially available equipment will have the necessary performance; this book will certainly help him to understand manufacturers' specifications but considerable skill and experience in electronics is necessary to solve those problems for which equipment is not available. Second, and especially for those with inadequate budgets, it is necessary to know how to build circuits with a performance which, though less than 'state of the art', may still be adequate for a particular purpose. So it seems a pity that Dr Nicholson, by concentrating on the best published circuits, gives little indication of what may be achieved more simply. For example, it would have been worth stressing how easy it is, even for the non-

specialist, to use digital integrated circuits in constructing counting equipment.

Despite such omissions, this book will be very useful to anybody working with nuclear counting systems. I noted only a few errors or misprints and the author is to be particularly commended on the clarity of his explanations and of the diagrams.

J. C. BARTON

Counting heads

A Workbook in Demography. By Roland Pressat. Translated by E. Grebenik with C. A. M. Sym. Pp. 292. (Methuen: London, May 1974.) £7.90.

THE age of the population explosion and its implications for society and for the world's resource base has attracted students in several disciplines to examine demographic problems. This interest is catered for by a growing general literature and by regional and case studies, but the techniques and methodology of demography have been generally somewhat neglected. Many postgraduate students find a need to provide their own basic training and regret the absence of straightforward, general textbooks that explain not only simply concepts but also the practical mechanism of calculating data from raw statistics, illustrating where relevant the major pitfalls for the unwary. Few are as well qualified to undertake this task as Professor Pressat, a mathematician by training, who has done much to define the aims and methods of the quantitative approach to population study. He has provided us with just such a textbook as described above, setting up problems, supplying suitable data and offering means of solution and explanation of significance.

The "exercises"—for this is truly a workbook—have been selected to present real life situations. The examples are spread widely over the confusing variety of situations that confront the beginner and the author brings to them an understandable order and relevance. For the more advanced student, he has sought to provide examples close to the situations faced by research workers. Not all the exercises are mathematical: several depend on descriptive interpretation. Nevertheless, using his early training, he makes the mathematics intelligible to those with only modest numeracy. Although designed for students in the University of Paris and consequently using substantial amounts of French data, the author has nevertheless selected from a wide range of countries, both "advanced" and "developing", and he has included some most interesting Soviet material.

The topics are grouped conceptually,

starting with the concept of a population. The reader is then taken through the measurement and comparison of mortality and some of the main problems of such difficulties as the misstatement of age. The exercises include not only contemporary situations but also the techniques of calculating data for past conditions. Anomalies, wherever they occur, are explained at length and in his answers the author makes some interesting comments on contemporary problems. For example, the stress of modern urban living may kill the weaker older people who survived infancy through the advances of modern medicine, whereas in earlier times they would have been winnowed by natural selection in childhood. Nuptiality, disunion (separation?) and re-marriage are dealt with in a similar manner and are followed by examination of fertility, where material is drawn from North American and African experience and used in comparative studies. The final section covers ageing population, infant mortality, birth order and replacement. It is a pity there is nothing on migration.

Treatment is thorough and wherever necessary basic techniques are explained. The student will find the many valuable formulae most useful and the author explains how to use fragmentary and unsatisfactory data by skilful manipulation. This is indispensable as a reference book for anybody interested in population.

R. E. H. MELLOR

Not so fast

Chemical Kinetics: Homogeneous Reactions. By N. M. Emanuel and D. G. Knorre. Translated from Russian by R. Kondor. Translation edited by D. Slutzkin. Pp. xii+447. (Wiley: New York and Toronto; Israel Program for Scientific Translations: Jerusalem, February 1974.) £8.

THIS is a soundly written book with a broad coverage of topics. It is, however, mainly concerned with work up to and including the early transition state era. As a succinct example, the work of Michael Polanyi on energy surfaces is described, but not that of his son John Polanyi. All this gives the book an oddly out-of-date flavour. Short references to flash photolysis, electron paramagnetic resonance and so on indicate the updating efforts of the second Russian edition (Moscow, 1969) but the general effect still remains. Honours students may look at the book for background material, chemists generally for the mathematical treatments of the concentration-time curves of various complex reactions. But there is nothing here comparable to the general monographs of, say, Kassel or

Hinshelwood—nothing to inspire the research workers in chemical kinetics, to draw young people into the subject, or to give succour to those peris outside paradise, the workers in heterogeneous catalysis. This is, I fear, a rather dull book: a Moskvich rather than a Lotus Elan. D. D. ELEY

Turbulent plasmas

Plasma Astrophysics. By S. A. Kaplan and V. N. Tsytovich. Translated by D. ter Haar. Pp. xiii + 302. (International Series of Monographs in Natural Philosophy.) (Pergamon: Oxford and New York, November 1973.) £9.50.

THIS book follows on naturally from *An Introduction to the Theory of Turbulent Plasmas* by Tsytovich, bringing together references to many of the papers published by Russian workers in the last few years. In this work the authors are concerned primarily with radiation and particle acceleration by turbulent plasmas in the sun, pulsars, nuclei of galaxies, radio galaxies and quasars. The authors restrict their attention to weak turbulence where the energy density of plasma waves is much less than the plasma thermal energy density. They assert that strong turbulence decays on a time scale which is short compared with astrophysical times leaving the plasma in a state of weak turbulence. Recently discovered 'spiccons' in laboratory plasmas with wave energy densities large in comparison with the thermal energy density may eventually cast some doubt on the universal applicability of the weak turbulence assumption to astrophysical plasmas. Within the weak turbulence assumption the authors discuss three types of turbulence: 'heating turbulence', where the energy of plasma waves is dissipated through electron-ion collisions and Landau damping; 'acceleration plasma turbulence', where the wave energy is lost in accelerating particles; and 'radiation plasma turbulence' where low frequency plasma wave energy is lost by the generation of high frequency electromagnetic waves produced by the interaction of plasma with particles.

The book is divided into four chapters: the first reviews, with liberal reference to the literature, laboratory and theoretical developments in plasma turbulence. The second chapter deals with sporadic solar emission, for example, how electromagnetic waves may be generated by ion-sound turbulence. The third discusses how turbulent processes can lead to observable radiation in for example radio galaxies and quasars, and the book ends with a discussion of pulsar emission.

The authors consider that the most important causes of plasma turbulence,

at least in a narrow frequency range are: beam, ion drift, loss-cone and anisotropy plasma instabilities. To these one should probably now add parametric instabilities occurring in the wave zones of pulsars. It is not yet clear whether refractive index effects, incurred by requiring a dense plasma to act as a vehicle for the turbulence, will inhibit radiative processes involving relativistic particles. The turbulent reactor production of a cosmic-ray ion power low spectrum meets with some difficulty if located in pulsars. The authors prefer to have the source in the nucleus of the galaxy; the implication for isotropy is not clear from their arguments.

The book is clearly written and the translator is obviously familiar with the scientific content of the work. The book is a very important addition to the astrophysicist's library; the new graduate student might however consider reading a more elementary text before attempting it. P. STEWART

Classifying vegetation

Ordination and Classification of Communities. Edited by R. H. Whittaker. Pp. x + 738. (Handbook of Vegetation Science, Vol. 5.) (Junk: The Hague, 1973.) Dfl. 160.

A DECADE ago the production of a volume such as this would not have been possible, but through the agency of the International Society for Plant Geography and Ecology symposia, the different approaches to vegetation, each with its own recondite peculiarities have been interchanged, discussed and dissected. But this is only half the story. To coax and cajole nineteen eminent practitioners from eight countries to contribute to the tome is no mean feat, and the praise for the commonplace drudgery and exasperating mechanics of coordination of the contributions falls firmly upon the energetic frame of Robert Whittaker. There can be no doubt that the dynamic personality of this man and the depth of scientific respect which he commands made the project a viable one, and the patient understanding provided by the publishers helped to ensure a production of the highest quality.

The book is divided naturally into three sections—direct gradient analysis, indirect gradient analysis and classification. The first section contains five papers, four of which have Whittaker as senior author, and a fifth written by Sobolev and Utekhin of the Soviet Academy of Sciences, Moscow. Much of Whittaker's contribution seems to be a new presentation of his earlier works, particularly his 1967 *Biological Review* paper, but as a part of a

general overview of methodology, its presence is vital. The two Russian authors provide a refreshing interpretation of the much abused Ramensky continuum concept which makes possible a far deeper insight into this approach than has been previously possible.

The second section contains typically thorough and informative papers on sample similarity and species correlation by Goodall, factor analysis by Dagnelie and ordination by resemblance matrices contributed by Orloci. The methods of comparative ordination evolved by the Wisconsin school some twenty years ago are re-examined by Cottam, Goff and Whittaker and, like the paper on matrix and plexus techniques by McIntosh will win many plaudits for its simplicity and clarity of presentation. But pride of place in this section must be given to the invaluable paper of Whittaker and Gauch which provides a realistic and erudite evaluation of ordination techniques using such criteria as efficiency, relative freedom from distortion of sample relationship and computational expenditure.

Whittaker is also responsible for two chapters in section 3—one, his familiar enumeration of the bases of vegetation classification and the different traditions which have evolved, the other a short discussion on the nature of dominance types which neatly complements the paper on the physiognomic approach by Beard. To many European phytosociologists, chapters 15 to 18 and 20 will prove to be invaluable, if not the highlight of the book. They comprise papers written by practitioners from various European countries who are fully conversant with the intricacies of and interpretations to be derived from methods which they themselves have used extensively. In this respect, the contributions of Frey, Alexandrova and Trass and Malmer on the Finnish, Russian and Scandinavian/Baltic approaches respectively help to fill a comparatively large gap in readily available literature in the English language. The synusial approach to classification is admirably dealt with by Barkman who covers a wide range of applications from epiphyte societies to aquatic vegetation types. In their exposition of the Braun-Blanquet methods, Westhoff and van der Maarel provide what is perhaps the best insight into the methodology in all its facets that has yet been written in English. A contribution on numerical taxonomy from Goodall completes this comprehensive section and the book.

There can be no doubt that this work will become one of the ecological classics. It is already my *vade mecum vegetatio*. D. W. SHIMWELL

obituary

J. Bronowski

DR JACOB BRONOWSKI died on August 21, 1974, aged 66. A mathematician, he won acclaim for his work in popularising science in books, on radio and in television broadcasts.

Bronowski was born in Poland and came to Britain in 1920. He read mathematics at the University of Cambridge from 1927 to 1930 and continued research there until 1933. A senior lecturer at the University of Hull from 1934 to 1942 he left to head a number of statistical units dealing with the effects of bombing. In 1945 he wrote the classic report *The effects of the Atomic Bombs at Hiroshima and Nagasaki*.

He was engaged in applying mathematical analysis to the economics of industry until 1950 when he became Director of the Coal Research Establishment of the National Coal Board and was responsible for the development of a new process for making smokeless fuel. In 1964 he joined the

Salk Institute for Biological Studies in San Diego, becoming Director of the Council for Biology in Human Affairs in 1970.

Bronowski published many books including *Science and Human Values*, and *The Western Intellectual Tradition*. He was a popular broadcaster and his most recent television series was the award winning *The Ascent of Man* made for the BBC.

L. C. Craig

LYMAN C. CRAIG whose invention of the counter-current distribution process has been particularly useful for the isolation and study of synthetic antimalarials, antibiotics, hormones and proteins died on July 7, 1974.

Dr Craig began his structural studies on the alkaloids of ergot in 1933, when he came to The Rockefeller University as an assistant to Dr W. A. Jacob, and later isolated lysergic acid. During the Second World War he worked on the chemistry of antimalarial drugs for

the Office of Scientific Research and Development.

R. Cruickshank

PROFESSOR ROBERT CRUICKSHANK, CBE, died on August 16, 1974 aged 74. He was prominent in forging many links between academic and public health microbiology.

He graduated from the University of Aberdeen in 1922 and, in 1928, was appointed Lecturer in Bacteriology at the University of Glasgow and Bacteriologist to Glasgow Royal Infirmary. Eight years later he became one of the first group pathologists of the London County Council and, in 1945, was appointed Director of the Central Public Health Laboratory Service. He succeeded Alexander Fleming as Professor of Bacteriology at St Mary's Hospital in 1948 and became Director of the Wright Fleming Institute of Microbiology. From 1958 until his retirement in 1966 he was Professor of Bacteriology at the University of Edinburgh.

Announcements

Appointment

G. R. Serjeant has been appointed director of the Medical Research Council's Epidemiology Unit in Jamaica.

International meetings

September 23–27, **22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy**, Manchester (D. W. Yalden, Organiser, 22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy, Department of Zoology, The University, Manchester M13 9PL, UK).

October 2–5, **International Conference on Surgery, Traumatology, Angiology Anaesthesiology and Reanimation**, Budapest (Office for Conference Organisation, (MOTESZ) H-1361 Budapest, POB 32, Hungary).

October 3–6, **6th International Symposium on Clinical Pharmacology and Annual Meeting of the International Society of Clinical Pharmacology**, Regensburg (International Symposia on Clinical Pharmacology, General Office,

D-8400 Regensburg 1, PO Box 345, German Federal Republic).

October 7–12, **International Exhibition and Symposium on Elements and Systems for the Preparation, Collection and Transmission of Data and other Peripheral Units of Computers: DATOS 74**, Bratislava (House of Technology of the Slovak Scientific and Technological Society, Bratislava, Kocelova 17, Czechoslovakia).

October 14–16, **2nd International Symposium on Oncogenesis and Herpes-Virus**, Erlangen (Dr H. zur Hausen, Institut für Klinische Virologie, Erlangen, Germany).

October 16–18, **6th Meeting of the International Society of Paediatric Oncology**, Genoa (Sixth Meeting of the International Society of Paediatric Oncology, Department of Paediatric Haematology and Oncology, Inst. Giannina Gaslini, via 5 Maggio 39, 16418 Genova-Quarto, Italy).

October 21–25, **International Conference on the Properties of Hydrazine and its Potential Applications as an Energy Source**, Poitiers (Centre Nat-

ional d'Etudes Spatiales (CNES), Département des Affaires Universitaires, 129 rue de l'Université, 75327 Paris Cedex 07, France).

October 27–November 1, **8th Pan American Congress of Endocrinology**, Buenos Aires (Secretariat, CENI S.A., Avda. Roque Saenz Pena 1110, Casillo de Corroero 2539, Buenos Aires, Argentina).

October 28–November 2, **International Conference on Plasma Theory**, Kiev (Professor Sitenko, Institute of Theoretical Physics, Metrologicheskaya St, 14-Kiev, USSR).

November 4–7, **5th European Meeting of Radioisotope Producers**, Athens (P. Papadimitropoulos, Director External Relations Divisions, Greek Atomic Energy Commission, Nuclear Research Center, 'Demokritos', Aghia Paraskevi Attikis, Athens, Greece).

November 4–8, **3rd International Symposium on Critical Care**, Rio de Janeiro (Dr Brenildo Tavares, Director, International Symposia on Critical Care, C. Postal 14700, ZC-95, Rio de Janeiro, Guanabara, Brazil).

November 4-13, **9th International Scientific Congress on the Cultivation of Edible Fungi (Part 1)**, Tokyo (9th International Scientific Congress on the Cultivation of Edible Fungi, Dr K. Ono, c/o Secretariat, RN606, Second Mitsui Building, 12-chome Muromachi, Nihonbashi, Chuo-ku, Tokyo, Japan).

November 5-6, **London Symposium on Radioimmunoassay**, London (Scientific Symposia Ltd., 121 King Street, London W6 9JG).

November 6, **Electron Beam Effects at Surfaces**, London (The Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

November 9-11, **5th Symposium on Plasma Physics and Controlled Nuclear Fusion Research**, Tokyo (International Atomic Energy Agency, P.O. Box 590, A-1011, Vienna, Austria).

November 15-18, **9th International Scientific Congress on the Cultivation of Edible Fungi (Part 2)**, Taipei (Dr Ren-jong Chiu, JCRR, 37 Nan Hai Road, Taipei, Taiwan).

November 20-25, **Symposium on Foetal Antigen Expression in Cancer**, Isawa, Japan (Dr E. Alpert, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, USA. or Dr H. Hirai, International Research Group for Carcino-embryonic Antigens, Hokkaido University Medical School, Sapporo, Hokkaido, Japan).

November 21-22, **Symposium on Nutrition and Aging**, New York (Director, Institute of Human Nutrition, Columbia University, 511 West 166th Street, New York, N.Y. 10032).

November 26-28, **Conference on the High Temperature Reactor and Process Applications**, London (British Nuclear Energy Society, Institution of Civil Engineers, 1-7 Great George Street, London SW1P 3AA).

Erratum

In the article "Structure of yeast phenylalanine tRNA at 3 Å resolution" by J. D. Robertus *et al.* (*Nature*, **250**, 546; 1974) the last sentence of the legend to Fig. 6 should read 'The region marked f is explained in the text as region 6.'

Reports and Publications

Great Britain

Fourth Annual Report of the Advisory Committee on Oil Pollution of the Sea Research Unit on the Rehabilitation of Oiled Seabirds. Pp. 27. (Newcastle-upon-Tyne: Department of Zoology, The University, 1974.) [17]
Public Health Laboratory Service. Monograph Series No. 4: The Distribution and Identification of Non-Fermenting Bacteria. By J. J. S. Snell. Pp. vii + 45. (London: HMSO, 1973.) 50p net. [37]
Does Research threaten Privacy or Does Privacy threaten Research?—Report of a Study Group. (Publication No. 74/1). Pp. 23. (London: British Association for the Advancement of Science, 1974.) 50p (40p to members.) [37]

Enquiry 1974. 10th Anniversary Edition. (The Science Journal of Harrow County School for Boys.) Pp. 112. (Harrow: Sales Manager, *Enquiry*, The County School for Boys, 1974.) 40p + 17p postage. [47]
Cancer Research Campaign. 51st Annual Report 1973, Incorporation Research Supported During 1974. Pp. 157. (London: Cancer Research Campaign, 2 Carlton House Terrace, 1974.) [47]
Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 5, No. 6: Contributions to the International Geodynamics Project. Pp. 87-122 + plates 11-14. £1.50. Vol. 5, No. 8: The Occurrence of Cordierite and Staurolite in a Part of the Leinster Granite Aureole: a Textural Study. By P. S. Kennan. Pp. 131-136. 30p. Vol. 5, No. 9: The Graignemanagh Belt in the Leinster Granite. By J. C. Brindley. Pp. 137-144. 30p. Vol. 5, No. 10: The Variable Granites at the Northern End of the Leinster Pluton. By J. C. Brindley and L. N. Gupta. Pp. 145-158 + plate 16. 40p. Series B. Vol. 3, No. 17: A Survey of Drinking Waters of Northern Ireland with particular reference to Their Fluoride Content. By C. McKay. Pp. 221-249. £1. (Dublin: Royal Dublin Society, 1974.) [47]
Mathilda and Terence Kennedy Institute of Rheumatology. 7th Annual Report 1973. Pp. 80. (Hammersmith, London W6: The Mathilda and Terence Kennedy Institute of Rheumatology, 1974.) [87]
Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1262: Seismicity and Structure of the Zagros (Iran)—the Main Recent Fault Between 33° and 35°N. By J. S. Tchalenko and J. Braud. Pp. 1-25 + plates 1-3. (London: The Royal Society, 1974.) [87]
Research Grants in the Natural Environment Sciences: NERC Research Grant Support Current Between 1 June 1972 and 31 May 1973. Pp. v + 102. (London: Natural Environment Research Council, 1974.) [87]
Scottish Plant Breeding Station. Fifty-third Annual Report, 1973/74. Pp. 84. (Pentlandsfield, Roslin, Midlothian: Scottish Plant Breeding Station, 1974.) [97]
National Radiological Protection Board. NRPB-R20: The Risk of Death from Radiation-Induced Cancer as Estimated from the Published Data on the Japanese Atomic Bomb Survivors. By S. G. Goss. Pp. 26. NRPB-R21: Assessment of Contamination from the Release of Activated Sulphur Hexafluoride from a Q-Tube Neutron Generator Used for Radiotherapy. By R. P. Rowlands and D. L. O. Humphries. Pp. 10. (Harwell, Didcot: National Radiological Protection Board, 1974.) [97]
Advisory Committee on Oil Pollution of the Sea. Annual Report for 1973. Pp. 28. (London: Advisory Committee on Oil Pollution of the Sea, 39 Victoria Street, 1974.) [107]

Other Countries

Final Report, Conference of Ministers of African Member States Responsible for the Application of Science and Technology to Development, organised by Unesco with the co-operation of the Economic Commission for Africa and the Organisation of African Unity. Dakar, 21-30 January 1974. Pp. 68. (Paris: Unesco, 1974.) [216]
Records of the Australian Museum. Vol. 29, No. 2: The Garfishes (Hemiramphidae) of Australia and New Zealand. By Bruce B. Collette. Pp. 11-105. (Sydney: The Australian Museum, 1974.) \$4. [256]
Democratic Republic of the Sudan. Ministry of Agriculture—Agricultural Research Corporation. Annual Report of the Gezira Research Station and Substations, 1968/1969. Pp. xi + 338. (Wad Medani: Gezira Research Station, 1974.) [256]
Smithsonian Contributions to the Earth Sciences. No. 12: An Atlas of Volcanic Ash. By Grant Heiken. Pp. iv + 101 (33 plates). \$2. Smithsonian Contributions to Zoology, No. 156: A Revision of North American *Capitophorus* Van der Goot and *Plectrotrichophorus* Börner (Homoptera: Aphididae). By L. A. Corpuz-Raros and Edwin F. Cook. Pp. iv + 143. \$2.25. (Washington, DC: Smithsonian Institution Press, 1974.) For sale by US Government Printing Office. [256]
CERN—European Organization for Nuclear Research. CERN 74-10: On Preconditioning and Convergence Acceleration in Sparse Matrix Problems. By O. Axelsson. Pp. v + 21. CERN 74-12: A General-Purpose Amplifier and Read-Out System for Multiwire Proportional Chambers. By J. Lindsay, Cl. Millerin, J. Cl. Tarle, H. Verweij and H. Wendler. Pp. 11. (Geneva: CERN, 1974.) [256]
Office de la Recherche Scientifique et Technique Outre-Mer, Bondy et Paris. Collection Travaux et Documents ORSTOM. No. 26: Étude des Mécanismes Physiologiques de la Formation des Sclérotos chez le *Corticium rolfsii* (Sacc) Curzi. Par M. Coujon. Pp. 107. 40 francs. Travaux et Documents ORSTOM No. 33: Le Bassin d'Ambalavao. Influence Urbaine et Evolution des Campagnes (sud Betsileo Madagascar). Par M. Portais. Pp. 172 + 8 planches. 66 Francs. Collection Initiations—Documentations Techniques No. 23: Initiation à la Morphologie, La Systématique et la Biologie des Insectes. Par M. Roth. Pp. 213 + 44 planches. 38 francs. Mémoires ORSTOM No. 69: Contribution à la Connaissance Physiologique de l'Homme noir dans Milieu Ecologique. Etude de l'Africain du Cameroun. Par C. Cavalier. Pp. 144. 60 francs. Mémoires ORSTOM No. 71: Les Euphausiacés du Pacifique Equatorial et Sue Tropical—Zoogéographie, Ecologie, Biologie et Situation Trophique. Par C. Roger. Pp. xx + 265. 100 francs. (Paris et Bondy: ORSTOM, 1974.) [256]
US Department of the Interior: Geological Survey Professional Paper 731: Geology of the Pulga and Bucks Lake Quadrangles, Butte and Plumas Counties, California. By Anna Hietanen. Pp. v + 66 + 3 plates,

\$3.45. Professional Paper 768: Lithostratigraphy and Depositional Environments of the Lexington Limestone (Ordovician) of Central Kentucky. By Earle R. Cressman. Pp. iv + 61 + 11 plates. \$8.70. (Washington, DC: Government Printing Office, 1973.) [266]
Smithsonian Contributions to Zoology, No. 161: Five New Bomolochid Copepods Parasitic on Indo-Pacific Clupeid Fishes. By Roger F. Cressey and Hillary Boyle. Pp. 25. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 60 cents. [266]
CERN—European Organization for Nuclear Research. CERN 74-13: Dipole Septum Magnets. By R. L. Keizer. Pp. 28. (Geneva: CERN, 1974.) [266]
Indian Forest Bulletin No. 267 (NS) (Forest Pathology): Microbial Degradation of Pulpwood. By B. K. Bakshi and Surjan Singh. Pp. 17. (Delhi: Manager of Publications, 1973.) RS. 1; 12p; 36 US cents. [266]
Forest Research in India. 1961-62, 1962-63, & 1963-64, Part 2: Reports from Indian States. Compiled by K. B. L. Mathur. Rs. 5; 58p; US\$1.80. 1964-65. Pp. 59. Rs.1.85; 22p. 67 US cents. (Delhi: Manager of Publications, 1972 and 1973.) [266]
Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Fisheries and Oceanography, 1972/1973. Pp. 67. (Cronulla, NSW: CSIRO, 1974.) [276]
Smithsonian Contributions to Zoology, No. 168: Ecology of the Squash and Gourd Bee, *Peponapis pruinosa*, on Cultivated Cucurbits in California (Hymenoptera: Apoidea). By Paul D. Hurd, Jr., E. Gordon Linsley and A. E. Michelbacher. Pp. iii + 17. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 60 cents. [276]
Royal Ontario Museum. Life Sciences Occasional Papers, No. 25: A New Subspecies of the Bat *Eumops auripendulus* (Chiroptera: Molossidae), from Argentina and Eastern Brazil. By Judy L. Eger. Pp. 8. (Toronto: Royal Ontario Museum, 1974.) [286]
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Annual Summary of Information on Natural Disasters: Earthquakes, Tsunamis, Volcanic Eruptions, Landslides, Avalanches, No. 7, 1972. Pp. 106. (Paris: The Unesco Press, 1974.) [17]
Bulletin of the Fisheries Research Board of Canada, No. 185: Benthic Errantiate Polychaetes of British Columbia and Washington. By Karl Banse and Katharine D. Hobson. Pp. x + 112. (Ottawa: Information Canada, 1974.) \$4.50. [17]
The Enterprise, Wisconsin, Radiation Forest: Preirradiation Ecological Studies. Edited by Thomas D. Rudolph. (TID-26113). Pp. v + 150. (Oak Ridge, Tennessee: US Atomic Energy Commission, Technical Information Center, 1974. Available from National Technical Information Service, US Department of Commerce, Springfield, Virginia 22151, USA.) \$7.60. [17]
Reports of the Finnish Geodetic Institute. No. 74-2: Time Keeping Methods Applied in Finland. By K. Kalliomaki and J. Kakkuri. Pp. 37. (Helsinki: Finnish Geodetic Institute, 1974.) [17]
Anticancer Agents Recently Developed in the People's Republic of China—A Review. By C. P. Li. (A Publication of the Geographic Health Studies, John E. Fogarty International Center for Advanced Study in the Health Sciences.) (US Department of Health, Education and Welfare Publication No. (NIH)74-441.) Pp. x + 255. (Washington, DC: Government Printing Office, 1974.) \$2. [17]
A New Look at Supplements: Casting Off Old Prejudices. (Based on a presentation of safety and efficacy submitted to the Food and Drug Administration OTC Review Panel on Vitamins, Minerals and Hematinics.) Pp. 14. (Washington, DC: Council for Responsible Nutrition, 1776 K Street NW, 1974.) [17]
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Low Level Lead Toxicity and the Environmental Impact of Cadmium. (*Environmental Health Perspectives* Experimental Issue No. 7, May 1974.) Pp. iv + 323. (National Institute of Environmental Health Sciences, National Institutes of Health, Education and Welfare.) (Washington, DC: US Government Printing Office, 1974.) \$3.05. [27]
Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-15: Publications on the Geology of the Interior Plains of Canada North of 60° Latitude by the Geological Survey of Canada. Compiled by E. Findlay. Pp. iii + 23. \$2. Paper 74-27: Geochemical Studies in the Eastern Part of the Slave Structural Province, 1973. By E. M. Cameron and C. C. Durham. With a contribution on the Petrology of the Volcanic Rocks by Mariette Turay. Pp. 22. \$3.50. (Ottawa: Information Canada, 1974.) [27]
Abhandlungen der Sachsichen Akademie der Wissenschaften zu Leipzig, Band 52, Heft 4: Beiträge zur D- und V-Struktur von Wasser und Speziellen Wassrigen Elektrolytösungen. Von Manfred Klose. Pp. 63. (Berlin: Akademie-Verlag, 1974.) 16 M. [27]
Radiation Protection Considerations on the Design and Operation of Particle Accelerators: Compilation prepared by Bengt G. Pettersson. Pp. 80. (Paris: Nuclear Energy Agency, OECD, 1974.) [37]
Smithsonian Contributions to Zoology, No. 157: A Bibliography of the Catalogs, Lists, Faunal and Other Papers on the Butterflies of North America North of Mexico Arranged by States and Province. (Lepidoptera: Rhopalocera). By William D. Field, Cyril F. Dos Passos and John H. Masters. Pp. 105. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.70. [37]